UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS RURAIS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA DO SOLO

DESTINO DO CARBONO DE RAÍZES E PARTE AÉREA DE CULTURAS DE INVERNO ENRIQUECIDAS COM ^{13}C EM SOLO SOB PLANTIO DIRETO

TESE DE DOUTORADO

Majid Mahmood Tahir

Santa Maria, RS, Brasil

2015

DESTINO DO CARBONO DE RAÍZES E PARTE AÉREA DE CULTURAS DE INVERNO ENRIQUECIDAS COM ^{13}C EM SOLO SOB PLANTIO DIRETO

Majid Mahmood Tahir

Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Ciência do Solo, Área de Concentração em Biodinâmica e Manejo do Solo, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de
Doutor em Ciência do Solo.

Orientador: Prof. Dr. Sandro José Giacomini

Santa Maria, RS, Brasil

2015

Ficha catalográfica elaborada através do Programa de Geração Automática
da Biblioteca Central da UFSM, com os dados fornecidos pelo(a) autor(a).

Tahir, Majid Mahmood
Destino do carbono de raízes e parte aérea de culturas de inverno enriquecidas com 13C em solo sob plantio direto. / Majid Mahmood Tahir.-2015.
145 p.; 30cm

Orientador: Sandro José Giacomini
Coorientador: Celso Aita
Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências Rurais, Programa de Pós-Graduação em Ciência do Solo, RS, 2015

1. Marcação com pulsos de 13C 2. Distribuição do 13C 3. Mineralização do 13C 4. Decomposição 5. Retenção de C no solo I. Giacomini, Sandro José II. Aita, Celso III. Título.

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Universidade Federal de Santa Maria
Centro de Ciências Rurais
Programa de Pós-Graduação em Ciência do Solo

A Comissão Examinadora, abaixo assinada,
aprova a Tese de Doutorado

DESTINO DO CARBONO DE RAÍZES E PARTE AÉREA DE CULTURAS DE INVERNO ENRIQUECIDAS COM ^{13}C EM SOLO SOB PLANTIO DIRETO

elaborada por
Majid Mahmood Tahir

como requisito parcial para obtenção do grau de
Doutor em Ciência do Solo

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Santa Maria, 28 de setembro de 2015.

DEDICATÓRIA

To My family

and

to

My wife Sadia

and our kids

Aiza

Ayesha

and

Salar

who gaves me stregth and reason to move on.....

لب پہ آتی ہے دعا بن کے تمنا میری
زندگی شمع کی صورت ہو خدایا میری

دُور دنیا کا مرے دم سے اندھیرا ہو جانے
ہر جگہ میرے چمکنے سے اُجالا ہو جانے

ہو مرے دم سے یونہی میرے وطن کی زینت
جس طرح پھول سے ہوتی ہے چمن کی زینت

زندگی ہو مری پروانے کی صورت یا رب
علم کی شمع سے ہو مجھ کو محبت یا رب

ہو مرا کام غریبوں کی حمایت کرنا
دردمندوں سے ضعیفوں سے محبت کرنا

مرے اللہ !برائی سے بچانا مُجھ کو
نیک جو راہ ہو اس رہ پہ چلانا مجھ کو

(علامہ اقبال)

Dos lábios vem uma oração na forma de um desejo
Minha vida seria emular uma vela, Oh Deus

Levante a escuridão mundana devido a meus esforços
Cada local deve ser iluminado pela minha Luz

Minha vida deve decorar a minha Pátria
Semelhante a uma flor que decora um jardim

Oh Deus, imitar a minha vida como um vaga-lumes
Eu cair no amor com a vela do Conhecimento

Meu trabalho deve ser apoiar os pobres
Aqueles que sofrem e os fracos eu deve amar

Meu Deus me salvar de fazer as coisas ruins
Por favor me orientar, para o caminho certo

(Dr. Allama Iqbal)

"Soil organic matter is the fuel that runs the soil's engine" (FISCHER, 1995)

AGRADECIMENTOS

I am thankful to God for bringing me in right place in right time among right people which helped me to achieve my doctoral degree.

I am deeply indebted to TWAS, Trieste, Italy and CNPq, Brazil for providing me the opportunity and financial assistance to come and realize my PhD in Brazil. I also thank The University of Poonch, Rawalakot, Azad Kashmir, Pakistan for granting study leave to pursue my PhD.

I wish to express my sincere thanks to my supervisor Prof. Dr. Sandro J. Giacomini for his guidance, encouragement and valuable support throughout the entire period of my study. I do not remeber any moment during my stay in Santa Maria when i needed his help and he was not there. As a good friend and mentor he made this experience enjoyable and memorable.

I would like to thank my co-supervisor Prof. Dr. Celso Aita for time to time help during the entire period and for all the advice and guidance during the writing of this thesis.

I would like to thank Dr. Sylvie Recous, for a warm welcome at INRA, Reims, France and for her incredible amount of time and energy during the write-up of this thesis. I am so grateful to work with such a cultured, knowledgeable and insightful lady. Many thanks to researchers and workers who welcomed me to work at INRA-Reims.

Thanks are due to Prof. Dr. Carlos A. Cerreta, for his helpful contributions along the way. Infact he is the man whose one click made UFSM my destination. He is such a gentle person ever known to me.

I am grateful to the committee members, Prof. Dr. Telmo Jorge Carneiro Amado, Prof Dr. Cimelio Bayer and Prof. Dr. Geferson Diekow who accepted to dedicate their precious time to evaluate this work.

A big thanks to Dr. Marciel Redin for the construction of labeling chmabers and to Dr. Eduardo Lorensi for the construction of wet sieving apparatus and cylinders insertion. Many

thanks to Andre Friderichs and Fabiano Helmenschlager for isotopic analyses. This work could not be completed without them.

I am also thankful to Prof. Dr. Paulo Ivonir Gubiani for his help in installing temperature and mositure sensors and help with calculations.

I will forever be indebted to the LABCEN where I worked and found many excellent colleagues and friends. I am grateful for the friendship and support provided by Stefen, Doneda, Eziquel, Cantu, Wesley, Gonzatto, Giacomini, Getúlio, Guilherme, Alex, Ricardo, Pedro, Heitor, Ismael, Bruno, Willian, Cledir, Michael, Raquel, Mariana, Lethicia, Carolina, Adriane, Roberta, Andre and Alessandra. I thank them from the bottom of my heart.

Thanks to the staff of the Department of Soils, Eunice, Héverton, Finamor, Paulinho, Pozzobom and Michel for their help and support.

Memories of my stay at Santa Maria will remain a treasure of my life. Thanks to Mohsin Zafar, Qamar Sarfaraz and Dr. Bhanudas Naik for their company.

Words are not simply enough to thank my wife Sadia for taking all the responsibilities back at home in Pakistan and for her patience, support and understanding.

My four years stay at UFSM was a great experience of learning and personal developement but i will always regret that i missed the joy of seeing my kids growing.

Finally, I would like to thank my parents, brothers and sisters for their unconditional love and support throughout my life and study here as a PhD student at the UFSM. I would never have got anywhere near this stage without such a great family.

A todos, MUITO OBRIGADO!

RESUMO

Tese de Doutorado
Programa de Pós-Graduação em Ciência do Solo
Universidade Federal de Santa Maria

DESTINO DO CARBONO DE RAÍZES E PARTE AÉREA DE CULTURAS DE INVERNO ENRIQUECIDAS COM ^{13}C EM SOLO SOB PLANTIO DIRETO

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ORIENTADOR: SANDRO JOSÉ GIACOMINI
Local e Data da Defesa: Santa Maria, 28 de setembro de 2015.

Pouco é conhecimento sobre a decomposição e a estabilização no solo do carbono (C) proveniente da parte aérea e de raízes *in situ* em sistema de plantio direto (SPD). O presente estudo foi desenvolvido com os seguintes objetivos: 1) avaliar o método de marcagem de plantas com ^{13}C através da aplicação de pulsos de ^{13}C no enriquecimento e distribuição do ^{13}C nas plantas cultivadas em condições de campo para posterior estudo da dinâmica de C; 2) estimar as taxas reais de mineralização do C de raízes *in situ* e da parte aérea na superfície do solo; e 3) determinar o destino do C da parte aérea e de raízes no solo, em condições de campo em SPD. O experimento foi conduzido na Universidade Federal de Santa Maria, Rio Grande do Sul, Brasil, em 2013-2014 em um Argissolo vermelho distrófico arênico. Plantas de Trigo (*Triticum aestivum* L.), ervilha (*Pisum sativum* L.) e ervilhaca (*Vicia sativa* L.) foram cultivadas em condições de campo dentro de cilindros de PVC, nas quais foram aplicados semanalmente pulsos de ^{13}C até o estágio de floração. A biomassa da parte aérea e das raízes, a composição química e o enriquecimento isotópico foram determinados no momento da colheita das plantas. A fim de alcançar o segundo objetivo, para cada cultura foi montado tratamentos pareados combinando a parte aérea marcada com ^{13}C com as raízes sem marcação e a parte aérea não marcada com ^{13}C com as raízes marcadas. Além desses foi utilizado um tratamento controle. Para o terceiro objetivo, os cilindros com solo foram escavados depois de 60, 180 e 365 dias após a instalação dos tratamentos pareados e realizada a determinação da distribuição nos agregados do C_{novo} derivado da parte aérea e das raízes. A composição química das plantas (parte aérea e raízes) não foi modificada pela marcagem com ^{13}C. O nível máximo de enriquecimento de ^{13}C nas plantas, no momento da colheita, foi de 495‰ no trigo, 426‰ na ervilha e 378‰ na ervilhaca. Os resultados demonstraram a heterogeneidade na distribuição do ^{13}C entre as partes da planta, particularmente entre caules e folhas, no entanto, esse nível de heterogeneidade é inferior aos reportados na literatura para experimentos de campo e em condições controladas. A mineralização de C das raízes das três espécies foi maior do que aquela observada para os resíduos da parte aérea (73 x 45 % no trigo, 76 x 48 % na ervilha e 73 x 51 % na ervilhaca). O ^{13}C remanescente nas raízes e na matéria orgânica do solo (MOS) aos 180 dias indicou elevada decomposição das raízes e alta taxa de C derivado das raízes na MOS comparado à parte aérea. Maior proporção do C_{novo} derivado da parte aérea e das raízes das três culturas foi associada aos macroagregados (>2000 µm) na camada de 0-5 cm, a qual diminuiu com o passar do tempo. O C derivado das raízes e da parte aérea nos microagregados (53–250 µm) aumentou gradualmente com o passar do tempo em todas as camadas do solo para todas as culturas. Aos 365 dias, 30% do C das raízes estava presente no solo, comparado aos 5% (média das três culturas) do C da parte aérea. A contribuição relativa média do C_{novo} derivado da raiz x parte aérea foi de 2,1 variando de 1,5 (ervilha) a 2,5 (trigo). Os resultados do presente trabalho sugerem que a localização dos resíduos culturais, o contato com o solo e a umidade e a temperatura do solo, são fatores importantes que promovem maior decomposição das raízes *in situ* e C_{novo} das raízes no solo, comparado com a parte aérea, reduzindo o efeito das diferenças na composição química inicial. A técnica de enriquecimento das plantas através da aplicação de pulsos de ^{13}C em condições de campo parece ser viável em relação à demanda de recursos é adequada para a marcagem *in situ*. Este trabalho fornece informações de suporte para estudos futuros, com enfoque nas interações entre os resíduos culturais da parte aérea e raízes e os fatores ambientais em condições de campo em SPD.

Palavras-chave: Marcação com pulsos de ^{13}C. Distribuição do ^{13}C. Mineralização do ^{13}C. Decomposição. Retenção de C no solo.

ABSTRACT

Doctoral Thesis Soil Science
Graduate Program in Soil Science
Federal University of Santa Maria, Brazil

FATE OF ROOTS AND SHOOTS CARBON OF WINTER CROPS LABELED WITH ^{13}C IN SOIL UNDER NO TILLAGE

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Date and Place of Defense: Santa Maria, September 28, 2015.

Little is known about the decomposition and stabilization of shoots and intact roots derived carbon (C) under no-tillage (no-till) field conditions. The present study was designed with following objectives: 1) evaluation of ^{13}C pulse labeling method to label crop plants under field conditions for subsequent C dynamics studies, 2) estimation of the actual rates of mineralization of intact roots and shoot residues, decomposing simultaneously, and 3) finally to determine the fate of shoot *vs* root residues derived C in soil, under no-till field conditions. The experiment was conducted at Federal University of Santa Maria , Rio Grande de Sul, Brazil in 2013-2014 in an loam textured Typic Paleudalf. Wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.) and vetch (*Vicia sativa* L.) plants were grown inside polyvinyl chloride (PVC) cylinders and were pulse labeled weekly with ^{13}C in the field until the flowering stage. At plant harvest, the biomass of shoots and roots and chemical composition and isotopic enrichment was determined. In order to achieve second objective, paired treatments were designed by combining ^{13}C labeled shoots with unlabeled roots+soil and unlabeled shoots with ^{13}C labeled roots+soil for each crop, plus a control treatment. For the third objective, soil cylinders were excavated after 60, 180 and 365 days for the determination of distribution of shoot and root derived Cnew in soil aggregates. The chemical composition of plant tissues (shoot and roots) were not modified by ^{13}C labeling. The maximum level of ^{13}C enrichment in plants at harvest, was +495‰ in wheat, +426‰ in pea and +378‰ in vetch plants. Our results though demonstrated heterogeneity of ^{13}C among plant parts particularly between stems and leaves however, it was far less than reported in other field and controlled conditions experiments. The mineralization of roots+soil C was higher than shoot-C residues for the three species (73 vs. 45 % initial C for wheat, 76 vs. 48 % for pea and 73 vs. 51 % for vetch). Remaining ^{13}C in root and soil organic matter (SOM) at day 180 indicated both a higher rate of root-C decomposition and a higher rate of root- derived C in SOM compared to shoots. Greater proportion of the shoot and root derived C_{new} of three crops was associated with large macroaggregates (>2000 µm) in 0-5 cm soil layer which declined with time. The content of root and shoot derived C microaggregates (53–250 µm) increased gradually with time in all the three soil for all crops. After 365 days, 30% of the root derived C was present in soil compared to 5 % (average of three crops) of the shoot derived C. The mean relative contribution of root *vs* shoot derived C_{new} was 2.1 ranging from 1.5 (pea) to 2.5 (wheat). Our findings suggest that, crop residues location and contact with soil and, the soil moisture and temperature, are important factors that significantly promoted roots decomposition and root derived C in soil *in situ*, compared to shoots, erasing the consequences of their different initial chemical composition. The $^{13}CO_2$ labeling technique used under the field appeared to be a practical approach with respect to resource demand and is suitable for *in situ* labeling. This work provides a framework for further studies focusing on the interactions between aboveground and belowground crop residues and environmental factors under no-till field conditions.

Keywords: ^{13}C pulse labeling. ^{13}C distribution. ^{13}C mineralization. Decomposition. C retention in soil.

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1 INTRODUÇÃO GERAL

O solo é uma importante fonte de emissão de dióxido de carbono (CO_2), um gás do efeito estufa causador do aquecimento global com consequências sobre as mudanças climáticas (JANSEENS et al., 2003). Estima-se que os solos do mundo emitem cerca de 20% do total das emissões de CO_2 através da respiração do solo (IPCC, 2007). Tal perda de C do solo está relacionada com a conversão dos ecossistemas naturais em agrícolas e com a prática de aração e gradagem no sistema convencional de preparo do solo (LAL, 2011). Os solos nesse sistema são consideravelmente pobres em C, o que tem conduzido a um interesse nos últimos anos em relação ao potencial global de diminuição dos solos aráveis (LAL, 2007). Uma vez que no solo encontra-se importante fração da quantidade total global do C e quase o dobro de C que está na atmosfera (1500 Pg C em solos, comparado a 760 Pg C na atmosfera), pequenas mudanças na armazenagem de C no solo podem causar um profundo impacto na concentração de CO_2 na atmosfera. Estima-se que um aumento na concentração de matéria orgânica do solo (MOS) em 5–15% até 2 m de profundidade no solo, poderia reduzir a concentração do CO_2 da atmosfera em 16–30% (KELL, 2011). Portanto, estudos sobre a estabilidade da MOS se torna um importante foco para pesquisas futuras.

A estabilidade da MOS e a armazenagem de C no solo são fortemente afetadas em primeiro lugar pelas práticas de manejo do solo (SMITH et al*.,* 2000). O preparo convencional do solo através da perturbação física promove a oxidação microbiana do C do solo (AL-KAISI; YIN, 2005). Por outro lado, a eliminação do preparo do solo geralmente resulta em decomposição mais lenta dos resíduos culturais e um aumento líquido no estoque de C no solo. O aumento de C no solo em sistema de plantio direto (SPD) é o resultado de dois principais fatores (1) formação de macroagregados ricos em C e (2) redução na taxa de conversão (*turnover*) dos microagregados (SIX et al., 2000). Os microagregados protegem fisicamente o C da decomposição microbiana por criar um microambiente anaeróbico, assim reduzindo a velocidade de decomposição da MOS, a qual concomitantemente aumenta o tempo de residência média do C no solo (SIX et al*.,* 2002). Diversas pesquisas reportam um aumento no estoque de C pela adoção do SPD em áreas agrícolas em todo o mundo (WEST; POST, 2002). Similarmente, resultados positivos também têm sido reportados no Brasil em condições de solos subtropicais, após a conversão do sistema convencional para SPD (BAYER et al., 2002; CARVALHO et al., 2009; ZANATTA et al., 2007). Acredita-se,

portanto, que a adoção do SPD preserva o C do solo, no entanto a preservação do C nesse sistema não é sempre observada (SANTOS et al., 2011). Isto sugere que a adoção do SPD não é uma pratica que funciona isoladamente e que depende de outros fatores como o tipo de cultura, a qualidade dos resíduos e fatores abióticos como a temperatura e umidade.

Os resíduos culturais são a principal fonte de MOS e sabe-se que a qualidade do resíduo pode afetar o armazenamento de C no solo. COTRUFO et al. (2013) sugerem que a qualidade dos resíduos das plantas afeta a taxa de estabilização da MOS através de sua influência na biomassa microbiana e no substrato usado. Esses autores apontam que os resíduos de plantas que aumentam a biomassa microbiana promoverão maiores oportunidades de estabilização da MOS. Nessa hipótese os resíduos de alta qualidade promovem aumento na eficiência microbiana resultando em menor produção de CO_2 e maior quantidade de resíduos microbianos por unidade de C metabolizado consequentemente maior quantidade de C pode ser retida no solo. Ao contrário, com resíduos de baixa qualidade menor é a eficiência dos microrganismos e menos resíduos microbianos são produzidos por quantidade de C metabolizado. Como resultado, os estoques estáveis de MOS são tidos como relativamente pequenos. BODDEY et al. (2010) observaram que o acúmulo de C na sucessão trigo-soja foi menor no SPD do que no solo preparo convencional. Nesse mesmo estudo foi observado que quando a planta de cobertura ervilhaca foi incluída na rotação, o acúmulo de C em SPD aumentou comparado ao solo em preparo convencional. Outros estudos também reportaram aumento nos estoques de C em SPD quando uma leguminosa foi incluída como cultura de cobertura na rotação de culturas (DIEKOW et al., 2005b; VIEIRA et al., 2009).

Além das diferentes culturas, o tipo de resíduos de culturais (parte aérea ou raízes) também influencia no sequestro de C nos solos. As diferenças na qualidade dos resíduos da parte aérea e raízes são importantes e resultam em distintas taxas de decomposição e estabilização de C no solo. Estas diferenças são ainda mais importantes em SPD devido a diferença na localização dos resíduos das culturas, por exemplo, os resíduos da parte aérea são mantidos na superfície do solo e as raízes no interior do solo. Décadas atrás, foi sugerido que o C das raízes influencia mais o balanço de C no solo do que os resíduos da parte aérea na superfície do solo (BROADBENT; NAKASHIMA, 1974). Em seguida, diversos estudos reportam uma estabilização preferencial do C das raízes, comparada ao C da parte aérea resultando em maior contribuição para o C do solo (RASSE et al., 2005). A maior estabilização do C das raízes foi proposta devido a (1) proteção física das raízes finas nos agregados do solo; (2) sorção dos exsudados das raízes ou decomposição de produtos em

superfícies minerais devido às suas proximidades; e (3) a natureza recalcitrante dos resíduos das raízes, comparada aos resíduos da parte aérea (RASSE et al., 2005).

As raízes são conhecidas por ter maior conteúdo de lignina, comparado aos resíduos da parte aérea (BERTRAND et al., 2006; CARRERA et al., 2008) e este atributo foi frequentemente ligado a uma decomposição mais lenta das raízes do que da parte aérea (POTTHAST et al., 2010). Poucos estudos que têm comparado a decomposição dos resíduos da parte aérea e das raízes em condições reais de campo reportam resultados contraditórios. Raízes têm sido reportadas como de decomposição mais rápida do que os resíduos da parte aérea mesmo apresentando maior conteúdo de lignina (BUYANOVSKY; WAGNER, 1987; BLENIS et al., 1999). Estes resultados contrastantes sugerem que as diferenças nas condições abióticas que estão sujeitos os resíduos da parte aérea deixados na superfície e as raízes intactas no interior do solo podem influenciar a dinâmica de decomposição. As diferenças na localização no solo geram diferenças no ambiente físico-químico e nas comunidades decompositoras, que é o principal fator individual que controla a decomposição dos resíduos à parte da composição química dos resíduos (FUJII; TAKEDA, 2010).

Até agora pouca atenção tem sido dada ao impacto potencial das entradas de C via resíduos culturais da parte aérea e das raízes no acúmulo de C no solo, em parte devido às dificuldades envolvidas na quantificação e rastreamento do C das raízes. Isso porque há muito mais estudos sobre a decomposição de resíduos da parte aérea do que sobre a decomposição das raízes (ZHANG et al., 2008). Similarmente, em condições de campo, onde os resíduos da parte aérea e das raízes são decompostos simultaneamente, não é possível com os métodos tradicionais diferenciar a contribuição relativa de cada fonte para a MOS. Técnicas de marcação isotópica são ferramentas confiáveis para rastrear a dinâmica do C dos resíduos da parte aérea e das raízes no solo. O advento do enriquecimento das plantas com ^{13}C em campo tem mostrado potencial para o estudo da dinâmica no solo do C dos resíduos *in situ* (PUGET; DRINKWATER, 2001). Para a marcação isotópica a campo a aplicação de pulsos de ^{13}C é preferível em relação à técnica de marcação contínua por causa da sua facilidade de manuseio e simples instrumentação. A marcagem de plantas em condições de campo é uma tarefa complexa, mas é necessária, particularmente para evitar alterações nas características do resíduo, composição química e conteúdo de N introduzido pela marcação de plantas em condições controladas. Um dos principais desafios é alcançar a distribuição homogênea da marcação entre as partes da planta.

Entender os padrões de decomposição e o destino do C dos resíduos culturais é necessário para determinar por quanto tempo tal sistema pode reter C. Estudos sobre a

contribuição da parte aérea e das raízes das culturas para o C no solo são escassos (RASSE et al., 2005), particularmente em culturas com contrastante composição química e em SPD. O sul do Brasil é uma importante região agrícola, principalmente conhecida pela produção de soja, milho, arroz, sorgo, trigo e feijão preto. Cerca de 27 milhões de hectares (Mha) estão sob SPD (BODDEY et al., 2010). Diversos estudos reportam um aumento no sequestro de C em SPD, comparado ao sistema de preparo convencional do solo (SANTOS et al., 2011). No entanto, são inexistentes estudos de campo sobre a dinâmica de decomposição da parte aérea e de raízes *in situ* em SPD e as estimativas de contribuição da parte aérea e raízes são frequentemente limitadas aos índices de colheita e proporções entre raízes e parte aérea (SANTOS et al., 2011). Consequentemente, um entendimento mais aprofundado da dinâmica do C derivado dos resíduos das raízes e da parte aérea e as suas implicações para o manejo agrícola é necessário. Portanto, neste estudo, foi conduzido um experimento de campo para avaliar a dinâmica de decomposição dos resíduos da parte aérea e das raízes de três culturas de inverno e a sua contribuição para o acúmulo de C no solo em condições de campo em SPD.

1.1 Objetivo geral

O objetivo geral deste trabalho foi estudar o destino do C durante a decomposição dos resíduos da parte aérea e das raízes de três culturas de inverno em SPD em condições subtropicais.

1.2 Objetivos específicos

1) Medir a homogeneidade do enriquecimento do ^{13}C entre as partes da planta e em frações químicas, e o grau de enriquecimento do ^{13}C para subsequente estudos da dinâmica do C das raízes e da parte aérea.

2) Avaliar a dinâmica da mineralização do C dos resíduos da parte aérea e das raízes *in situ* marcadas com ^{13}C em condições de campo em SPD.

3) Avaliar durante a decomposição da parte aérea e das raízes de três culturas de inverno a contribuição para o acúmulo de ^{13}C em agregados do solo estáveis em água em SPD.

Nós formulamos as seguintes hipóteses, as quais fornecem a base para o trabalho de pesquisa conduzido para esta tese.

1.3 Hipóteses

1) A homogeneidade razoável da marcação de ^{13}C entre as partes das plantas em condições de campo pode ser alcançada através da aplicação de ^{13}C em intervalos sucessivos.

2) As diferenças na decomposição entre as raízes e a parte aérea, devido à sua composição química, podem ser reduzidas sob condições de campo em SPD, por causa de uma condição de decomposição mais favorável encontrada pelas raízes no interior do solo.

3) Os resíduos das raízes contribuirão com mais C para o solo do que os resíduos da parte aérea.

1.4 Abordagem da pesquisa

Os resultados reportados nesta tese são a continuação de um experimento iniciado a partir da marcação de plantas com ^{13}C (Artigo 1), seguido pela subsequente estimativa da mineralização de C da parte aérea e de raízes *in situ* (Artigo 2) e o destino do C da parte aérea e das raízes em diferentes classes de tamanho de agregados (Artigo 3), sob condições de campo em SPD. Seguem as abordagens utilizadas para realizar o experimento:

1) Cilindros foram inseridos ao solo para facilitar a amostragem das raízes e do solo e para evitar a contaminação dos resíduos marcados na superfície do solo com os resíduos não marcados.
2) Três diferentes culturas foram usadas para avaliar a influência do tipo de cultura sobre a dinâmica do C. As culturas selecionadas pertencem a diferentes famílias e são usadas para diferentes propósitos, por exemplo, a ervilhaca como uma cultura de cobertura e o trigo como cereal.

3) A marcação isotópica (^{13}C) das plantas cultivadas foi necessária para rastrear o C dos resíduos da planta através de várias associações do C. Portanto, nós ajustamos um sistema de marcação para marcar plantas sob condições de campo. Uma ilustração fotográfica do sistema de marcação e do crescimento das plantas dentro dos cilindros com solo é apresentada no Artigo 1 (Figura 2).

4) Para investigar a decomposição da parte aérea e das raízes e o destino do C derivado desses resíduos em diferentes compartimentos no solo foram montados para cada cultura tratamentos pareados combinando a parte aérea marcada com ^{13}C com as raízes não marcadas (LS) e a parte aérea não marcada com as raízes marcadas com ^{13}C (LR) (raízes + solo). Uma ilustração dos tratamentos pareados é apresentada no Artigo 2 (Figura 3).

5) As plantas foram marcadas no campo e as raízes não foram perturbadas. O solo onde cresceram as plantas marcadas continham ^{13}C (rizodeposição) anteriormente ao segundo experimento (Artigo 2), o que não foi o caso para as plantas não marcadas. Portanto, foi estimada a quantidade de ^{13}C a partir da rizodeposição e do C derivado das raízes, incluindo a decomposição dos rizodepósitos e das raízes (raiz + solo), enquanto o C derivado da parte aérea foi composto apenas pela decomposição dos resíduos da parte aérea.

6) Uma abordagem de fracionamento físico (tamisamento úmido) foi usada para avaliar a hipótese das mudanças na estabilização do C da parte aérea e raízes em diferentes classes de tamanho de agregados.

1.5 Visão geral da tese

Esta tese é estruturada em 5 principais capítulos, iniciando com a introdução geral, seguida por três artigos e uma discussão geral.

1) O primeiro artigo descreve a marcagem das plantas cultivadas sob condições de campo, a produção de matéria seca das plantas marcadas e não marcadas, a distribuição do ^{13}C nas diferentes frações da planta e o grau de enriquecimento em ^{13}C. Este artigo é preparado de acordo com o formato da revista *Nutrient Cycling in Agroecosystems.*

2) O segundo artigo descreve a dinâmica de decomposição dos resíduos da parte aérea e das raízes das três culturas sob condições de campo em SPD. Este artigo é preparado de acordo com o formato da revista *Soil Biology and Biochemistry*.

3) O terceiro artigo é sobre o destino e a estabilização do C derivado das raízes e da parte aérea em diferentes tamanhos de agregados de solo. Este artigo também descreve a

contribuição relativa do C derivado das raízes x parte aérea. Este artigo é preparado de acordo com o formato da revista *Plant and Soil*.

4) Uma discussão geral dos resultados compõe o quarto capítulo deste trabalho, seguido pelas considerações para futuras pesquisas, referências e apêndices.

2 ARTIGO I – Production of ^{13}C-enriched pea, wheat and vetch in the field during a sub-tropical winter for subsequent studies of residue decomposition[1]

2.1 Abstract

The pulse-labeling of crop plants provides a unique opportunity to trace the fate of decomposing residues in the soil-plant system. We evaluated a $^{13}CO_2$ pulse labeling method to label crop plants under field conditions for subsequent C dynamics studies. The experiment was conducted at the Federal University of Santa Maria (UFSM), Rio Grande de Sul (RS), Brazil. Wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.) and vetch (*Vicia sativa* L.) crops were grown inside polyvinyl chloride cylinders, which were hydraulically forced into the soil (30 cm deep). Pulse labeling was conducted in the field once a week for 11 weeks. The ^{13}C enrichment of plant parts and chemical fractions and the recovery and distribution of the ^{13}C in shoots, roots and soil was determined. Plant dry matter production and the chemical composition of plant tissues (above and belowground) were not modified by ^{13}C labeling. The maximum level of ^{13}C enrichment in plants at harvest, as expressed in $\delta^{13}C$, was +495‰ in wheat, +426‰ in pea and +378‰ in vetch plants. All three crops showed similar patterns of ^{13}C distribution with ^{13}C recovery in the shoots > roots > soil (0-30 cm). On average, 81 to 89 % of the recovered ^{13}C was in the shoots, 7 to 14% was in the roots, and 2.7 to 4.3% was recovered in the soil. The $^{13}CO_2$ labeling technique used under the field conditions allowed for the production of sufficiently labeled wheat, pea and vetch plant parts. Therefore, we believe that this is a practical approach with respect to resource demand (tracer and labor costs) that is suitable for *in situ* labeling.

Keywords: Isotopic homogeneity, Labeling uniformity, Plant parts, Chemical fractions, $^{13}CO_2$ pulse labeling

[1] Article is prepared in accordance with the format of Journal *Nutrient Cycling in Agroecosystems*.

2.2 Introduction

Crop residues are the primary source of all SOM and the type of crop residues influence C sequestration is soils. The differences in quality of above- and below-ground plant residues and in their location during decomposition under no-till system, result in different C mineralization and stabilization pathways, which are likely to contribute to soil C in different ways and amounts. It is therefore important to distinguish between these two component of plants residues for a better understanding of their fate in soil.

Isotopic labeling techniques are reliable tools for tracking above- and below-ground residue C dynamics in soil. In a literature survey, we found that most of the labeled residues that were used for C dynamics studies were labeled under controlled conditions (i.e. Aita et al. 1997; Sanaullah et al. 2010). This finding may call into question the actual estimates of C contributions to SOM, especially from root residues because of possible bias introduced in the root's decomposition due to removing, washing and drying them, which not only results in the loss of fine roots and soluble C but also destroys root-rhizosphere interaction (Aulen et al. 2012). Although it is a complicated task to label plants under field conditions (Comeau et al. 2013), it is necessary particularly where no-tillage (no-till) is practiced, to mimic actual field conditions of root (i.e. intact roots) and shoot decomposition.

The advent of field labeling has shown the potential to investigate C residue dynamics under actual field conditions (i.e., Puget and Drinkwater 2001). However, until the present, few field experiments have been reported with the purpose of studying the C dynamics of above and below-ground residues by using *in situ* labeling (i.e., Puget and Drinkwater 2001; William et al. 2006; Kong and Six 2010). For field isotopic labeling, pulse labeling is ideal relative to the continuous labeling technique because of its easy handling and simple instrumentation, but successful atmospheric labeling with $^{13}CO_2$ poses a number of challenges i.e. homogeneous distribution of label within the plants parts and chemical fractions and level of ^{13}C enrichment for subsequent detection. Bromand et al. (2001) suggested that if the label is applied at regular intervals that are frequent enough to represent C assimilation during the growing season, it may be possible to obtain adequate homogeneity for tracing plant residue decomposition through various C pools. The field labeling experiments that were developed in the past were mainly focused on the enrichment of residues with ^{13}C and none of the investigators checked how the applied C isotope was distributed within chemical fractions (Puget and Drinkwater 2001; Kong and Six 2010). It is therefore important to know how ^{13}C label was distributed among chemical fractions because different chemical fractions

decompose at different rates (Trinsoutrot et al. 2000) and heterogeneous labeling impact the interpretation of SOM analysis in subsequent decomposition studies (Sangster et al. 2010).

Therefore, the aim of this study was to set up and evaluate a pulse labeling method for labeling crop residues with ^{13}C under field conditions. The method was applied to three winter crops (wheat, pea and vetch) grown in soil cylinders under no-till field conditions. We hypothesized that the uniform distribution of the label in plant parts and biochemical fractions can be achieved by applying $^{13}CO_2$ pulses at regular intervals over the growing season. The objectives of the work were to assess (i) the homogeneity of ^{13}C enrichment among plant parts and in chemical fractions and (ii) the degree of ^{13}C enrichment for subsequent root C and shoot C dynamics studies. We also compared the dry matter and quality of crop residues between labeled and unlabeled plants to be able to use "paired" treatments in future decomposition studies.

2.3 Materials and Methods

2.3.1 Site description

The field study was conducted from May to October 2013 (autumn/winter) in the experimental area (29°41′ S, 53°48′ W; approximately 90 m elevation) of the Department of Soils, which is located at the Federal University of Santa Maria (UFSM), Rio Grande de Sul (RS) in southern Brazil. The experimental area has a humid subtropical Cfa type (Mild with no dry season, hot summer) climate according to the Koeppen classification (Koeppen 1948). The annual average temperature is 19.3°C, with the coldest monthly temperature below 9°C in June and the warmest above 30°C in January. The average annual precipitation is 1769 mm, without the dry season but highly variable. The mean daily air temperature and daily precipitation during the experiment is shown in Fig. 1. The soil at the study site was classified as Typic Paleudalf according to the USDA Taxonomy (Soil Survey Staff 2010), has a sandy loam A horizon with 10 % clay, 27% silt, 18 % fine sand, 63 % coarse sand, 1.5 g.cm^{-3} bulk density and 6.6 g C kg^{-1}and $\delta^{13}C$ of -19.17 in the top 0–30 cm soil layer. Prior to the labeling experiment, the site had been under no-tillage for 15 yr with a 2-year crop rotation with oat (*Avena sativa*) or vetch (*Vicia sativa*) during the winter season and corn (*Zea mays*) or soybeans (*Glycine max*)] during the summer season.

2.3.2 Experimental setup

On May 15, 2013, a 25 m × 25 m^2 area within the experimental site was marked, cleared and fenced for the labeling of three winter crops, namely wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.) and vetch (*Vicia sativa* L.). The experiment was designed according to a split-plot design with crops as a main plots and with, or without ^{13}C pulse labeling as sub-plots. Within a given plot, two sub-plots were arranged in a randomized complete block design (RCBD) with three replications (3 crops × 2 sub-plots × 3 replications = 18 sub-plots). The sub-plots were of the same dimensions as the labeling chambers (0.80 m × 0.80 m). One sub-plot was used for pulse labeling with ^{13}C (which is henceforth referred to as the "labeled sub-plot"), and the other was kept unlabeled (the "unlabeled sub-plot"). Inside each sub-plot, nine polyvinyl chloride (PVC) cylinders (hereafter, "soil cylinders") of 20 cm in diameter and 35 cm in length were hydraulically forced into a 30 cm soil depth. The soil cylinders were kept at 5 cm above the soil surface and they had small holes on each side for water drainage. In addition, a total of twelve soil cylinders were similarly forced into the soil in the area between sub-plots as controls without plants to serve as a reference for calculating the ^{13}C distribution in labeled sub-plots. On May 29, 2013, eight wheat, pea and vetch seeds were sowed in each soil cylinder. After emergence, the plants were thinned to four plants for wheat, two plants for pea and four plants for vetch in each cylinder. Before sowing, the soil was limed with dolomitic limestone at a rate of 2 Mg ha^{-1} to raise the pH from 4.7 to 5.5. Phosphorus and potassium were applied at a rate of 50 kg ha^{-1}. Nitrogen was applied by splitting 90 kg N ha^{-1} into six equal doses (15 kg N ha^{-1} per dose) for wheat, and it was applied in the form of ammonium sulfate. All other standard and cultural practices were performed during the whole experiment.

2.3.3 Labeling chamber specification

Modified portable chambers were used for *in situ* ^{13}C pulse labeling according to Sangster et al. (2010). Instead of using one large chamber, we used small chambers that could be adjusted according to the heights of the plants (Fig. 2. a, b). The labeling chambers were constructed with 2 mm-thick clear, ultraviolet light-transparent acrylic sheets mounted on iron frames. The labeling chambers consisted of two sections, i.e., an upper section and an iron base. The upper section was a box (0.80 m × 0.80 m·× 0.30 m) that was set on an iron base (0.80 m·× 0.80 m × 0.05 m). The bottom edge of this box was immersed in a water-filled trough (0.03 m

wide and 0.03 m high) on an iron base, thereby forming an airtight seal. In addition, an open-ended chamber extension (0.80 m × 0.80 m·× 0.30 m) was also made for raising the chamber height according to the growth of the plants. An axial 12 V DC fan (15 cm^2) was positioned centrally on the upper surface of the labeling chamber to promote air mixing and uniform heat distribution. Inside the chamber, a platform was constructed next to the axial fan to hold a plastic container. Above this platform, an injection port with a septum was fitted on each chamber. An infrared gas analyzer (IRGA, model SD800, Extech, USA) with a data logger was connected to the top of the chamber to monitor the total CO_2 concentration, temperature and humidity inside the chamber. The iron bases were installed around soil cylinders in each labeled sub-plot in the soil at a 5 cm depth before the onset of pulse labeling. The labeled and unlabeled sub-plots were kept apart from each other at a distance of 2 m to minimize any potential lack of independence during pulse labeling.

2.3.4 ^{13}C pulse-labeling

The pulse labeling was started on June 22 and was performed weekly thereafter until the plants had flowered. The ^{13}C-labeling events usually took place between 9:00 and 11:00 for a total of 1.5 h. The labeled sub-plots were enclosed inside labeling chambers, resting on iron bases and sealed with water. A 30 ml of 2 mol L^{-1} HCl was injected into the plastic container (100 mL) through the septum. The intended enrichment of CO_2 in the chamber was 33 atom% ^{13}C. Before every pulse labeling event, the diluting effect of unlabeled CO_2 inside the labeling chamber was compensated by injecting $^{13}CO_2$ (99 atom % ^{13}C). For this, CO_2 concentration was allowed to fall to 266 ppm by plant uptake. After the CO_2 concentration fell to 266 ppm, 0.580 moles of $NaH^{13}CO_3$ (99 atom % ^{13}C) was injected into the plastic container through the septum, yielding an estimated ^{13}C abundance in labeling chamber (192 L) air CO_2 close to the intended 33 atom%. When the height of the labeling chambers was raised to 384 L and 576 L with the growth of the plants, 1.16 and 1.74 moles of $NaH^{13}CO_3$ (99 atom % ^{13}C) were injected after CO_2 concentration was fell to 266 ppm. After the first injection, whenever the CO_2 concentration was dropped to 266 ppm, $NaH^{13}CO_3$ solution (33 % $NaH^{13}CO_3$ and 66% $NaH^{12}CO_3$) was injected into the plastic container containing an excess of HCl during 1.5 h to maintain a total CO_2 concentration between 266 ppm and 400 ppm. In this way, the frequency of injections increased proportionally with the rate of CO_2 removal and, by definition, $^{13}CO_2$ addition was therefore always proportional to photosynthesis, regardless of plant growth stage

(assuming all CO_2 removal was by photosynthesis). Ice packs were placed inside the chamber to minimize excessive heating and to condense excess humidity.

The CO_2 levels were monitored by the IRGA throughout the labeling period and average values were recorded every 20 seconds. Because of the difference in the wavelengths of $^{13}CO_2$ and $^{12}CO_2$, IRGA only provided an estimate of the total CO_2. After each labeling event, the chambers were replaced over labeled sub-plots at sunset to capture overnight $^{13}CO_2$ respiration, thereby improving ^{13}C enrichment (Kong and Six 2010). The chambers were removed the following morning after the CO_2 levels fell below 266 ppm. The wheat, pea and vetch plants were exposed to a total of 1.7, 2.0 and 1.8 g of $NaH^{13}CO_3$, respectively. The difference in amounts was related to differences in photosynthetic rates as well as differences in the growth stages.

2.3.5 Shoot, root and soil sampling

Wheat plants were harvested at early dough stage, pea plants at maturity and vetch plants were harvested at pod formation stage. All plants were harvested on the same day by clipping them at the soil surface and separating them into stems, leaves, pods/chaffs and grain. After the plants were harvested, three randomly selected soil cylinders from labeled and unlabeled sub-plots were excavated from the soil by digging down to 35 cm. In addition, three randomly selected control soil cylinders (without plants) were also excavated. The cylinders were then carefully removed, placed in plastic bags and transferred to the laboratory, and they were kept at 4°C and processed within 2 days. Each whole soil core (0-30 cm) was taken out by cutting the PVC cylinder at both sides with an electric saw. The soil cores were wrapped in plastic film to avoid any soil loss and then separated into three soil layers (0-5, 5-15 and 15-30 cm). The weight of each soil layer was recorded. All visible roots were immediately removed by hand from each soil layer; the soil of each layer was then thoroughly mixed, and 100 g moist soil was sub-sampled and suspended in 200 mL of deionized water. Fine roots were removed by gentle shaking and rinsed further with tap water until clean. After root collection, a sub-sample of soil was used for soil moisture. The remaining soil was passed through a 2-mm sieve, air-dried and systematically mixed to ensure representative sub-sampling. The soil was finely ground in a steel ball mill for the determination of total C and $\delta^{13}C$.

2.3.6 Chemical and isotopic analysis

Roots and shoots (stems, leaves, pods/chaff and grain) were sub-sampled in triplicate to determine the dry matter (DM), the total C and total N concentration, and ^{13}C atom excess and chemical composition (neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (LIG)). One sub-sample was oven-dried at 65°C for 48 h for DM correction. The second sub-sample was dried at 40°C and ground into 1 mm particles for chemical analysis. The third sub-sample was dried at 40°C, and it was first ground in a coffee grinder and then with a steel ball mill for C, N and $\delta^{13}C$ analyses. A proximate analysis was performed on two sets of stem, leaf and root sub-samples that were dried at 40 °C and then ground to a 1 mm particle length. One set was used for the determination of chemical fractions (soluble (SOL), cellulose (CEL), hemicellulose (HEM) and lignin (LIG)) and the other was used for the determination of C and $\delta^{13}C$. The chemical fractions were determined by Van Soest method (Van Soest 1963) as described by Redin et al. (2014a).

The total C and N contents of the roots and shoots (stems, leaves, pods/chaff and grain) and of their chemical fractions, neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (LIG) were determined by using an elemental analyzer (Flash EA 1112, Thermo Electron Corporation, Breman, Germany). Similarly, the total C and $\delta^{13}C$ of the soil, the $\delta^{13}C$ of the whole plant parts and of the NDF, ADF and LIG samples were measured with an elemental analyzer (Flash 2000, Thermo Electron Corporation, Breman, Germany) coupled to an isotope ratio mass spectrometer (Delta V Advantage, IRMS Thermo Fisher Scientific Inc. Germany). The total C and N contents and $\delta^{13}C$ values in the SOL, CEL and HEM fractions were then calculated.

2.3.7 Calculations

The isotopic values are expressed in relation to the Vienna-Pee Dee Belemnite (V-PDB) reference as $\delta^{13}C$ (Ge et al. 2012) as follows:

$$\delta^{13}C(‰) = \left(\frac{R_{sample}}{R_{V-PDB}} - 1\right) \times 1000 \quad (1)$$

$$^{13}C(\text{atom }\%) = 100 \times \frac{\left((\delta^{13}C+1000)\times R_{V-PDB}\right)}{\left((\delta^{13}C+1000)\times R_{V-PDB}+1000\right)} \quad (2)$$

$$^{13}C \text{ excess (atom \%)} = {}^{13}C \text{ sample(atom \%)} - {}^{13}C \text{ natural abundance (atom \%)} \quad (3)$$

where the R_{sample} is the $^{13}C/^{12}C$ ratio in the sample and the $R_{V\text{-}PDB}$ is equal to 0.011179. The amount of ^{13}C (mg) that was incorporated into the shoot, root and soil was calculated by using the following equation (An et al. 2015):

$$^{13}C_{\ amount} = C_{Sample} \times \left(A_{labeled\ sample} - A_{unlabeled\ sample}\right) \quad (4)$$

where C_{sample} refers to the C content (mg) of each portion; $A_{labeled}$ sample and $A_{unlabeled}$ sample refer to the ^{13}C atom % of labeled samples and unlabeled samples, respectively. The ^{13}C recovery (%) was expressed as a percentage of net fixed ^{13}C out of the total amount of added ^{13}C. The percentage of the ^{13}C label that was partitioned into the shoots, roots and bulk soil was expressed as a percentage of ^{13}C in the shoots, roots and bulk soil as the total amount of recovered ^{13}C.

2.3.8 Statistics

The experiment was implemented with a randomized complete block design with a split plot arrangement and three replications. The data of shoot and root biomass, the ^{13}C enrichment of plant parts and biochemical fractions of wheat, pea and vetch plants were analyzed by three-factor analysis of variance (ANOVA). Crop species and plant parts were used as independent variables and ^{13}C enrichment was used as a dependent variable. The LSD (least significance difference, ($P<0.05$) was used to test differences in variables between wheat, pea and vetch crops. All statistical tests were performed with STATISTIX 8.1.

2.4 Results

2.4.1 Plant dry matter

The mean shoot and root dry matter (DM) for labeled and unlabeled plants are given in Table 1. The shoot DM (excluding grain) ranged from 982 to 1129 g m^{-2} for wheat, 1009 to 1070 g m^{-2} for pea and 1185 to 1385 g m^{-2} for vetch plants. The mean root DM ranged from 129 to 134 g m^{-2} for wheat, 157 to 188 g m^{-2} for pea and 179 to 208 g m^{-2} for vetch plants. Labeled plants tend to have a slightly higher DM compared with unlabeled plants for all plant parts except vetch roots. The DM differences between shoot parts were only significant ($P > 0.05$)

for vetch leaves; the root dry weights were not significantly (P = 0.05) different between labeled and unlabeled plants. This finding indicates that ^{13}C pulse labeling did not affect the DM yield significantly and that labeled and unlabeled plants grew under similar conditions.

2.4.2. Plant total C and N and chemical composition

The total C concentration of the labeled and unlabeled shoots and roots of the three crops were not affected by labeling (Table 1). The total N concentration of the shoots showed a similar pattern (Table 1). The N content of the roots differed only for vetch roots that were sampled from the 15 to 30 cm soil layer. However, the total root N was unaffected by labeling when calculated over the whole cylinder depth.

The chemical composition (SOL, CEL, HEM and LIG) of the labeled plant parts (stems, leaves and roots) are presented in Fig. 3 (a, b, c). The labeled and unlabeled plant parts had similar compositions (data not shown). The three crops had higher SOL contents and lower LIG contents in the leaves compared with the stems and roots. Overall, the distribution of C in the biochemical fractions (SOL, CEL, HEM and LIG) followed the distribution of DM, and it did not differ significantly between labeled and unlabeled plants (Table 2). Some differences were found for the HEM-C in vetch stems and pea leaves, for the LIG-C in wheat stems and pea roots, and for the N in the LIG for vetch roots. Nitrogen primarily accumulated in the SOL fraction, ranging from 65 % in roots to 90 % in the stems and leaves of the total N present in the chemical fractions of the three crops (Table 2).

2.4.3 $\delta^{13}C$ enrichment of plant parts

The mean $\delta^{13}C$ enrichment of the plants increased significantly ($P < 0.05$) from -27 ‰ to 495 ‰ in wheat, -29 ‰ to 426 ‰ in pea and -29 ‰ to 378 ‰ in vetch plants as a result of ^{13}C pulse labeling (Table 3). The $\delta^{13}C$ enrichment of the stems and leaves differ significantly for wheat and vetch plants, and homogeneous $\delta^{13}C$ enrichment was obtained for the leaves and stems of pea plants. The pod and grain fractions were the least enriched. Higher ^{13}C enrichment was observed in the roots of the three crops compared with the aboveground parts (with the exception of wheat leaves), and the differences in the ^{13}C enrichments between soil layers were small. Among the crops, the ^{13}C enrichment of stems was not different (p = 0.103), and for the other plant parts, the enrichment was significantly different ($p < 0.05$) with the ranking wheat >pea >vetch. The ^{13}C was homogeneously distributed in the CEL, HEM

and LIG fractions of the wheat stems, leaves and roots given that the observed differences were not significantly different (Fig. 3d, e, f). The SOL fraction was least enriched regardless of the plant part and the crop under consideration, except in the vetch root.

2.4.4 ^{13}C recovery in plants and soil

An isotopic shift in the soil $\delta^{13}C$ values of the labeled sub-plots was observed after harvesting. The $\delta^{13}C$ value of the control soil was -19.17 ‰ which was increased to -16.73 ‰ in wheat, -17.15 ‰ in pea and -16.41 ‰ in vetch labeled sub-plots in 0-30 cm soil layer. A slight decrease in soil $\delta^{13}C$ values of the unlabeled sub-plots of the three crop was also observed when compared with control (-19.33, -19.28 and -19.48 ‰ in wheat, pea and vetch, respectively). The total C concentration in 0-30 cm soil layer after plant harvest was not affected and was similar to that in control soil. The recovery of ^{13}C from plants (above- and below ground parts) and bulk soil was 44.8 % of the total ^{13}C applied to wheat, 34.7 % to pea and 35.1 % to vetch from labeled sub-plots (Table 4). More than half of the recovered ^{13}C was found in the leaves and stems. Belowground ^{13}C was primarily root ^{13}C and averaged 5 % of the applied ^{13}C. The recovery of ^{13}C in the root fraction decreased with the decreasing root DM, i.e., with increasing soil depth. A small proportion of recovered ^{13}C was found in the bulk soil. This recovery was not significantly different between crops and among soil depths. On average, 81 to 89 % of the net recovered ^{13}C remained in the shoots, 7 to 14 % was incorporated into the roots, and 2.7 to 4.3 % was retained in the soil (Fig. 4). This amount of net fixed ^{13}C that was retained in the soil-plant system did not differ significantly between crops.

2.5 Discussion

2.5.1 Plant production and composition

This study was conducted in the field and involved the repeat pulse $^{13}CO_2$ labeling of three winter crops to obtain the above and belowground residues enriched with ^{13}C, and identical in terms of the chemical composition and DM to their counterpart above and belowground residues of the unlabeled plants. This was the key requirement to study simultaneous decomposition of above- and belowground residue C in soil, which could be done by combining labeled roots with unlabeled shoots and unlabeled roots with labeled shoots (paired

treatments). The differences in the chemical composition between labeled and unlabeled residues and in DM, particularly for roots, would not only jeopardize the subsequent residues decomposition studies but also lead to the misinterpretations of the results, if used. With the ^{13}C pulse method used here, the above and below-ground DM of labeled and unlabeled plants did not vary significantly and were generally consistent with previously reported results for repeat pulse labeling experiments (Bromand et al. 2001; Puget and Drinkwater 2001; Meng et al. 2013). However, the crop DM was higher compared with the DM production reported for similar crops under field conditions with similar climatic conditions (e.g. Boody et al. 2010). The higher plant production obtained in our experiment may be attributed to the better management of crops (i.e. the plant selection, stalking of pea and vetch plants, split fertilization, etc.). Additionally, leaves that senesced during the growth period (from vetch and pea) were not allowed to decompose, i.e. they were collected and included in the total DM, which is not the case in the field. We also acknowledge that the pre-mature harvesting of wheat plants also contributed to the observed higher DM of wheat plants compared to field plants. We were intended to use the above-and belowground residues of the three crops plants for subsequent decomposition studies at the same time under similar environmental conditions, which was our motive to harvest wheat plants before maturity. Therefore, we recommend to better consider the growth cycle of the different crops before the onset of the labeling, even this requires a more complex experimental design if several crops are labeled simultaneously, as it was the case in this study. The total C and N concentrations and the chemical composition of the plant parts were within the range reported for similar crops grown under actual field conditions (Redin et al. 2014 a, b) except the wheat crop, which had a higher N concentration. This high N concentration for wheat may be related to the split application of N fertilizer and a crop harvest in the early dough stage. Taken together, these findings indicate that labeled residues obtained by $^{13}CO_2$ pulse labeling could be used for future reciprocal treatment experiments (i.e. the exchange of residues between labeled and unlabeled plots) for tracing the root C compared with the shoot C in SOM pools simultaneously (e.g. Gale and Cambardella 2000).

2.5.2 $\delta^{13}C$ enrichment of plant parts

The plants showed higher $\delta^{13}C$ enrichment in our experiment compared to those of Puget and Drinkwater (2001), Williams et al. (2006), and Kong and Six (2010) under field conditions. The plants in these experiments were pulse labeled with 99 atom % $^{13}CO_2$ while the intended

atmospheric CO_2 enrichment in our experiment was 33 atom % ^{13}C. Therefore. the differences in final $\delta^{13}C$ enrichment of the plants show that the frequency of labeling events and the labeling duration are critical issues for designing pulse-labeling in the field. Under subtropical climatic conditions, the 1.5 h duration of labeling session appeared suitable for the enrichment of plant residues: though ice packs were used to prevent excessive heating of the labeling chamber above optimum for CO_2 uptake, we believe that duration longer than 1.5 h without a proper cooling system will not enhance ^{13}C enrichment. We also labeled plants between 9:00 and 11:00 am, to help in keeping labeling chamber temperature between 25 to 30°C. This may call in question the availability of sufficient photosynthetically active radiation (PAR) for photosynthesis compared to the labeling around midday or later. However, the ^{13}C enrichment of residues in our experiment was greater than other studies (Puget and Drinkwater 2001; Williams et al. 2006; Kong and Six 2010) where labeling was done between 10:00 am and 16: 00 pm, which suggest that labeling in early hours is possible. Another possible reason is the re-fixation of overnight-respired $^{13}CO_2$ and its investment in building depleted leaf starch reserves during the following morning. An important finding in our experiment that is not consistent with other studies (i.e. Puget and Drinkwater 2001; Kong and Six 2010; Sangster et al. 2010) is the higher enrichment of roots compared with aboveground residues. Bromand et al. (2001) reported similar results with wheat plants and described higher root enrichment resulting from a succession of labeling pulses and an effective translocation of ^{13}C to roots. As we started the labeling at the early stages of plant growth, we assumed that the translocated assimilates were efficiently used in the buildup of roots, leading to the higher enrichment of root tissues.

Likewise plant parts, complete homogeneity in the distribution of ^{13}C label among chemical fractions was not achieved, the ^{13}C label was mostly homogeneously distributed among the CEL, HEM and LIG chemical fractions of the leaves, stems and roots of the three crops. Plants and biochemical components within each individual plant already differ in their natural ^{13}C composition under natural atmospheric conditions. However, under repeat-pulse labeling conditions, heterogeneous enrichment of biochemical fractions is probably due to the relationship between developmental stage of the plants and labeling events (Sangster et al. 2010). Williams et al. (2006) concluded that the pulse-labeled materials could be used for C cycling studies despite the heterogeneous distribution of the ^{13}C label in biochemical fractions of clover and ryegrass. Because we used repeat pulses at regular intervals, the heterogeneous distribution of ^{13}C label in different plant parts and biochemical fractions was minimized.

This distribution could be further improved by considering a decrease in the time intervals between the labeling sessions while maintaining a reasonable experimental cost.

Besides the challenge of achieving homogeneous distribution of ^{13}C label in plant parts and chemical fractions under field conditions, another important question is whether the enrichment obtained was sufficiently great (roughly + 400 ‰) for use in subsequent decay studies. On average for the three crops, we found that the ^{13}C enrichments of the shoot residues obtained from our field labeling was 2, 1 and 0.17 times greater than reported by Williams et al. (2006), Puget and Drinkwater (2001) and Kong and Six (2010), respectively. The differences were even more prominent for root residues, where root ^{13}C enrichment in our experiment was 12 times greater than reported by Williams et al. (2006) and 5 times to those reported by Puget and Drinkwater (2001) and Kong and Six (2010). We believe that the number of pulses (11 compared to c.a. 5 in Puget and Drinkwater (2001); Williams et al. (2006); Kong and Six (2010), likely was responsible for greater ^{13}C enrichment of shoot and root residues in our experiment. This supports our analysis that increasing the number of pulses, e.g. twice a week would not only enhance the ^{13}C enrichment of the plant residues but also would result in an increased homogeneity of distribution of the label within plant parts and chemical fractions. Though, it will increase the cost of labeling but it will be far less than the cost of continuous labeling covering the entire growth cycle of the plants.

2.5.3 Recovery and distribution of ^{13}C

The amount of fixed ^{13}C remaining in the plant-soil system was relatively low, but it was comparable with the values reported in the literature in which plants were labeled and harvested after maturity (i.e. Wichern et al. 2007; Gregory and Atwell 1991). The relatively low recovery of applied ^{13}C was possibly related to respiration by shoots and roots and microbial respiration (Ostle et al. 2000). Most of the recovered ^{13}C was incorporated into the shoots. Gregory and Atwell (1991) also reported 91-95 % of recovered ^{14}C in wheat and barley shoots. In our experiment, the roots were generally more enriched than the shoots and the total amount of ^{13}C was low. This finding suggests that there was an active belowground routing of ^{13}C during the active root growth period and that it is reduced as the plant becomes mature. Lu et al. (2002) recovered more photosynthates in the earlier plant growth stage compared with the maturation stage. Additionally, a decrease in the root biomass during plant growth also resulted in lower ^{13}C accumulation in the roots (Gregory and Atwell 1991; Yevdokimov et al. 2006).

A minor proportion of the total ^{13}C was recovered in bulk soil (2.7 to 4.3 % of applied). Low bulk soil ^{13}C recovery after pulse labeling was also reported by Staddon et al. (2003) and Rangel-Castro et al. (2004). In our experiment, bulk soil ^{13}C enrichment after 40 days of the last labeling was within the reported range. Most of the ^{13}C assimilates entering the soil during plant growth via rhizodeposition are labile and are available for microbial respiration rather than stabilization. Derrien et al. (2004) estimated that 40 % of root-derived C from wheat was made of soluble neutral sugars, which were highly labile for microbial respiration. Gregory and Atwell (1991) found 33 % and 54 % of the total recovered ^{14}C in below-ground components in wheat and barley, and half was respired as $^{14}CO_2$ during the early stages of growth. Taken together, this finding may indicate that the nature of root-derived ^{13}C-enriched assimilates that were released into the soil and microbial mineralization strongly affected the recovery of ^{13}C in soil.

2.6 Conclusions

The repeat $^{13}CO_2$ pulse labeling technique used under field conditions was shown to be adequate for sufficiently labeling the wheat, pea and vetch C in the various above and belowground plant parts. Our results though demonstrated some heterogeneity in the ^{13}C distribution among plant parts, particularly between stems and leaves but this heterogeneity was far less than reported earlier in other field and controlled conditions experiments. Therefore the pulse-labeling method evaluated here have useful application in C cycling studies. The distribution of ^{13}C label in biochemical fractions was reasonably homogeneous with few exceptions. We found that *in situ* repeat-pulse labeling efficiency was comparable to other studies conducted under control conditions. Therefore, we believe that this is an appropriate approach with respect to resource demand (tracer and labor costs) and suitability for the *in situ* labeling of crops for subsequent field scale C turnover studies.

Acknowledgments

The first author's doctoral study was funded by CNPq-TWAS postgraduate fellowship programme. This work was supported by the Brazilian government through the Conselho Nacional de Desenvolvimento Científico e Tecnologico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS). The bilateral Brazilian and French collaboration was funded under Program CNPq—Ciência sem Fronteiras Process Number

401724/2012-3. CN-MIP project (ANR-13-JFAC-0001) provided a grant to M.M. Tahir for his leave at INRA, Reims, France. We thank Dr. Marciel Redin for assistance during labeling and André Friderichs for ^{13}C isotopic analysis.

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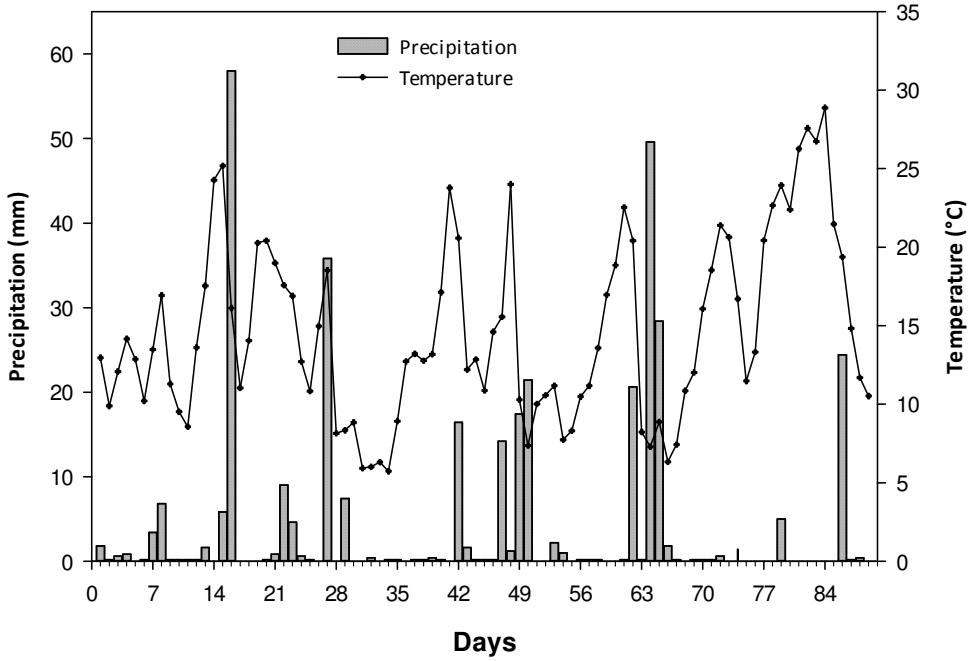

Fig. 1 Precipitation and mean daily air temperature during the labeling of crop plants under field conditions. Day 0 is start of experiment (22-06-2013)

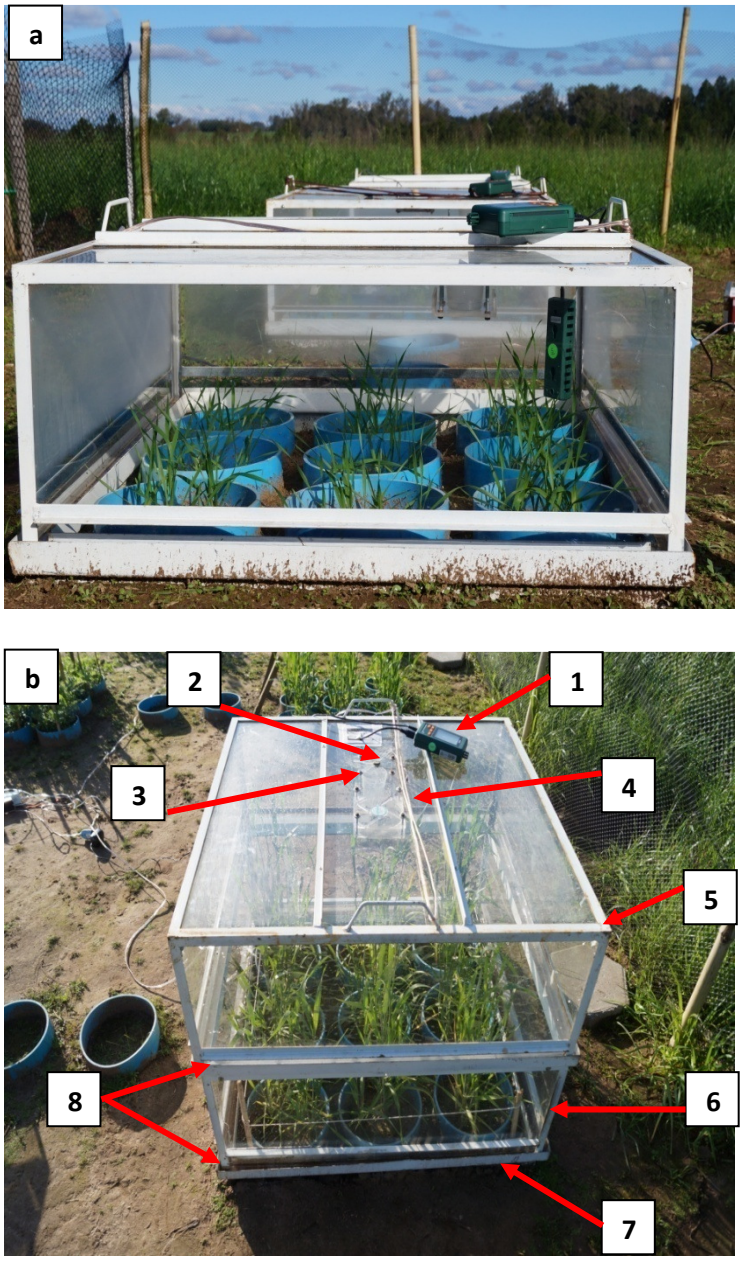

Fig. 2 Photographs of the labeled sub-plot with labeling chamber used for ^{13}C pulse labeling (a) labeling chamber and chamber extension (b). Components are, 1: IRGA, 2: rubber septum, 3:plastic container (HCL), 4: Fan, 5:labeling chamber, 6: chamber extension, 7: iron base, 8: water seal

Table 1 Average shoot and root biomass production at plant harvests and total C and N concentrations of labeled and unlabeled crop plants that were grown under field conditions

Crop plants	Shoots				Roots			
	Stem	Leaves	Chaff/Pods	Grain	0-5[a]	5-15	15-30	0-30
Dry matter (g m^{-2})								
Wheat-L	638.8a	145.4a	345.3a	267.0a	105.5a	16.3a	12.3a	134.1a
Wheat-U	550.4a	130.9a	274.6b	245.8b	95.8a	19.3a	14.7a	129.8a
Pea-L	618.4a	234.9a	216.5a	597.0a	43.7a	26.1a	24.3a	94.1a
Pea-U	566.4a	231.9a	210.8a	584.0a	40.3a	19.5a	19.0a	78.8a
Vetch-L	508.5a	796.7a	–	–	129.2a	35.5a	15.0a	179.7a
Vetch-U	481.8a	703.5b	–	–	145.5a	44.6a	18.7a	208.8a
Total C (g kg^{-1})								
Wheat-L	437.9a	435.1a	436.9a	431.3a	398.2a	415.3b	396.9b	400.1a
Wheat-U	436.0a	442.0a	437.6a	431.4a	395.9a	419.4a	413.5a	402.2a
Pea-L	445.1a	425.9a	421.0a	424.5a	420.1a	417.7a	420.7a	420.1a
Pea-U	437.0a	426.5a	421.4a	424.9a	442.7a	425.5a	426.0a	434.3a
Vetch-L	428.9a	424.3a	–	–	397.2a	399.3a	415.0a	399.2a
Vetch-U	431.9a	425.3a	–	–	390.5a	393.6a	404.1a	392.0a
Total N (g kg^{-1})								
Wheat-L	12.2a	24.7a	17.4a	26.6a	14.1a	17.0a	16.8a	14.7a
Wheat-U	11.7a	25.0a	17.7a	26.7a	14.0a	16.5a	16.3a	14.7a
Pea-L	14.8a	28.3a	20.1a	42.6a	32.1a	31.3a	29.3a	31.1a
Pea-U	14.6a	25.7a	19.9a	42.5a	33.3a	30.9a	32.3a	32.5a
Vetch-L	22.1a	40.5a	–	–	28.7a	35.8a	35.2a	30.6a
Vetch-U	18.7a	39.1a	–	–	30.3a	32.9a	31.0b	31.1a

[a]Soil depth in cm. Wheat-L: wheat labeled, Wheat-U: wheat unlabeled; Pea-L: pea labeled, Pea-U, pea unlabeled, Vetch-L: vetch labeled, Vetch-U: vetch unlabeled. The values are the means of 3 replicates. Numbers followed by different lowercase letters within a column represent significant differences at $P < 0.05$ between labeled and unlabeled plants for a given component

Table 2 Total C and total N concentrations of chemical fractions from labeled and unlabeled crop plant parts (stems, leaves, and roots) grown under field conditions

Crop plants	Total C (g C kg^{-1} DM)				Total N (g N kg^{-1} DM)			
	SOL	CEL	HEM	LIG	SOL	CEL	HEM	LIG
Stems								
Wheat-L	177.6a	128.3a	111.4a	20.5b	10.7a	0.4a	0.7a	0.2b
Wheat-U	162.3a	131.3a	117.4a	25.4a	10.1a	0.4a	0.8a	0.4a
Pea-L	130.3a	181.0a	79.2a	54.7b	12.8a	0.6a	0.3a	1.0a
Pea-U	125.9a	171.5a	76.3a	64.4a	12.4a	0.6a	0.4a	1.2a
Vetch-L	130.4a	174.9a	65.7b	57.8a	18.8a	0.8a	0.13a	1.1a
Vetch-U	131.6a	166.3a	74.1a	60.3a	15.4a	0.7a	0.11a	1.4a
Leaves								
Wheat-L	197.6a	118.0a	107.9a	9.8a	22.3a	0.6a	1.8a	0.2a
Wheat-U	201.9a	118.1a	112.5a	10.1a	22.6a	0.6a	1.5a	0.3a
Pea-L	283.8ab	98.5a	34.5a	9.2a	24.5a	0.1a	2.2a	0.4a
Pea-U	300.9a	92.1a	25.3b	9.0a	22.2a	0.1a	1.6b	0.4a
Vetch-L	278.2a	81.1a	45.5a	19.7a	36.6a	0.6a	2.2a	0.7a
Vetch-U	288.7a	83.9a	30.9a	21.7a	35.4a	0.8a	1.7a	0.7a
Roots								
Wheat-L	102.5a	156.3a	115.2a	26.1a	10.1a	2.8a	1.0a	0.8a
Wheat-U	104.7a	155.2a	117.8a	24.3a	10.1a	2.7a	1.1a	0.8a
Pea-L	136.5a	119.9a	104.1a	59.5b	20.6a	6.5b	1.1a	2.9a
Pea-U	136.0a	121.8a	107.1a	69.3a	20.2a	7.7a	0.6a	4.0a
Vetch-L	152.5a	140.3a	71.9a	34.3a	19.0a	8.4a	1.5b	1.7b
Vetch-U	155.2a	128.6a	73.1a	35.1a	17.3a	8.6a	2.2a	3.0a

DM: dry matter Wheat-L: wheat labeled, Wheat-U: wheat unlabeled; Pea-L: pea labeled, Pea-U, pea unlabeled, Vetch-L: vetch labeled, Vetch-U: vetch unlabeled. SOL: soluble fraction; CEL: cellulose; HEM: hemicellulose; and LIG: lignin. The values are the means of 3 replicates. Numbers followed by different lowercase letters within a column represent significant differences at $P < 0.05$ between labeled and unlabeled plants for a given component

Table 3 $\delta^{13}C$ (‰) values of labeled and unlabeled parts of crop plants grown under field conditions

Crop plants	Shoot				Root		
	Stem	Leaves	Pod	Seed	0-5[a]	5-15	15-30
	Labeled						
Wheat	357.4 aC	493.4 aA	328.2 aD	192.8 aE	495.4 aA	490.4 aA	453.9 aB
Pea	365.3 aB	365.7 bB	189.9 bC	124.3bD	417.6 bA	426.6 bA	413.1 bA
Vetch	315.1 aB	254.8 cC	–	–	378.7 cA	324.5 cB	318.6 cB
	Unlabeled						
Wheat	-27.1 bB	-29.5 dC	-29.6 cC	-28.4 cB	-26.5 dA	-28.0 dB	-28.0 dB
Pea	-30.3 bB	-29.3 dA	-30.1 cB	-29.2 cA	-30.5 dB	-30.5 eB	-30.2 dB
Vetch	-30.9 bC	-29.4 dB	–	–	-28.8 dA	-30.2 eC	-29.8 dB

[a] Soil depth in cm. Numbers followed by different lowercase letters within each column and uppercase letters within each row represent significant differences at $P < 0.05$ among labeled and unlabeled crops and between the plant parts of a given crop, respectively

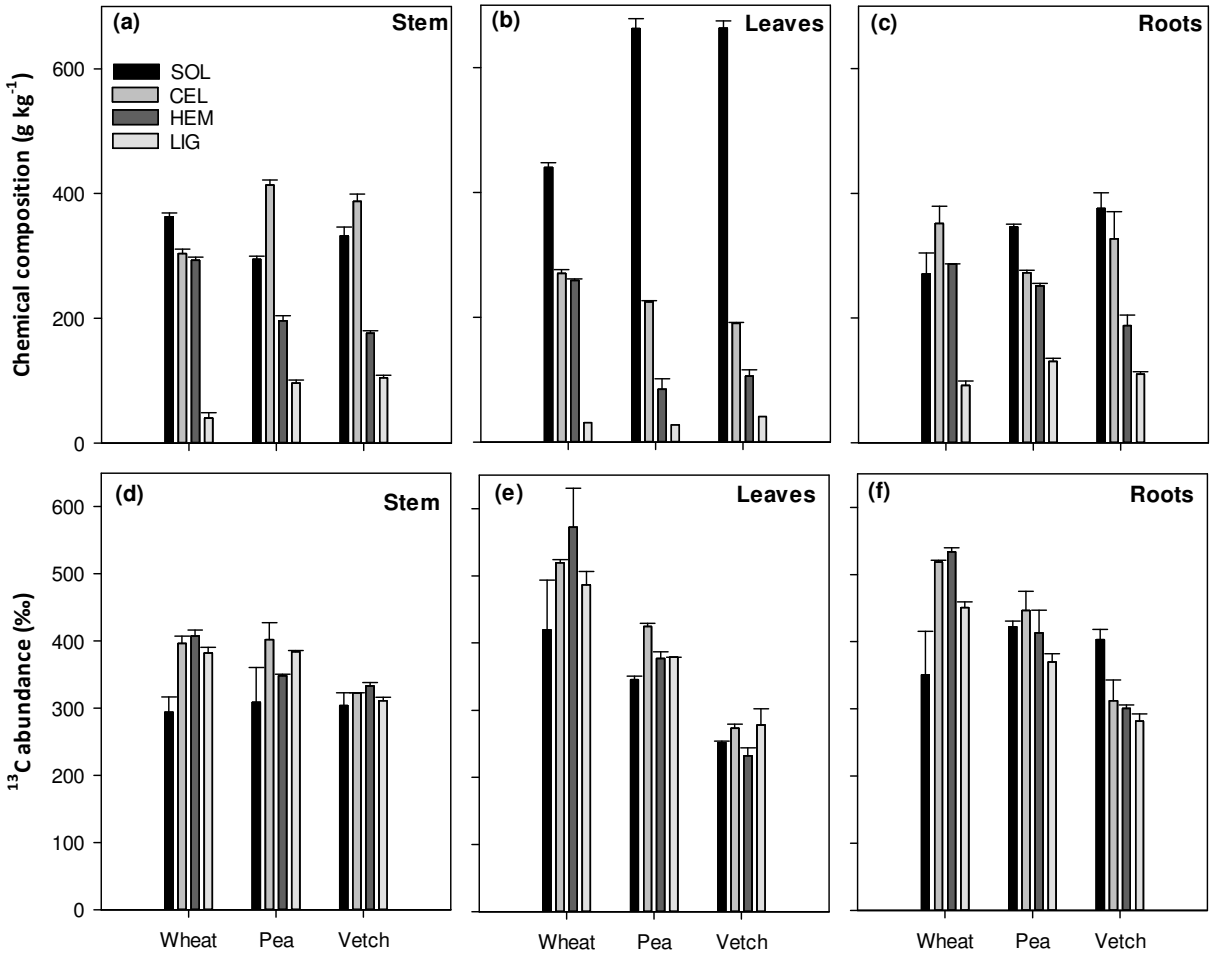

Fig. 3 Chemical composition (a, b and c) and $\delta^{13}C$ of chemical fractions (d, e and f) in the stems, leaves and roots of three crops grown under field conditions. Error bars indicate the standard error (n = 3). SOL = soluble, CEL = cellulose, HEM = hemicellulose, LIG = lignin

Table 4 Recovery (% of applied ^{13}C) and partitioning (% of recovered ^{13}C) in different parts of wheat, pea and vetch plants and within three soil depths after repeat-pulse labeling under field conditions

Crop	Shoots				Roots			Soil			
plants	Stem	Leaves	Chaff/Pod	Grain	0-5[a]	5-15	15-30	0-5	5-15	15-30	Total
	Recovery of ^{13}C										
Wheat	18.94a	5.84b	9.48a	4.49a	3.58a	0.62a	0.41a	0.81a	0.31a	0.35a	44.82a
Pea	16.21a	5.93b	3.02b	5.88a	1.23a	0.75a	0.68a	0.45a	0.19a	0.34a	34.69a
Vetch	12.47a	15.86a	–	–	4.06a	0.83a	0.36a	0.74a	0.42a	0.34a	35.07a
	Partitioning of ^{13}C										
Wheat	41.94a	12.93b	21.45a	10.12b	7.98a	1.33a	0.90b	1.84a	0.70a	0.80a	100
Pea	46.23a	16.70b	9.06b	17.64a	3.63b	2.10a	1.91a	1.33a	0.51a	0.89a	100
Vetch	35.98a	45.58a	–	–	10.98a	2.22a	0.97b	2.20a	1.16a	0.93a	100

[a]Soil depth in cm. Different lowercase letters within a column represent significant differences among crops of a given component for the amount of ^{13}C ($P < 0.05$)

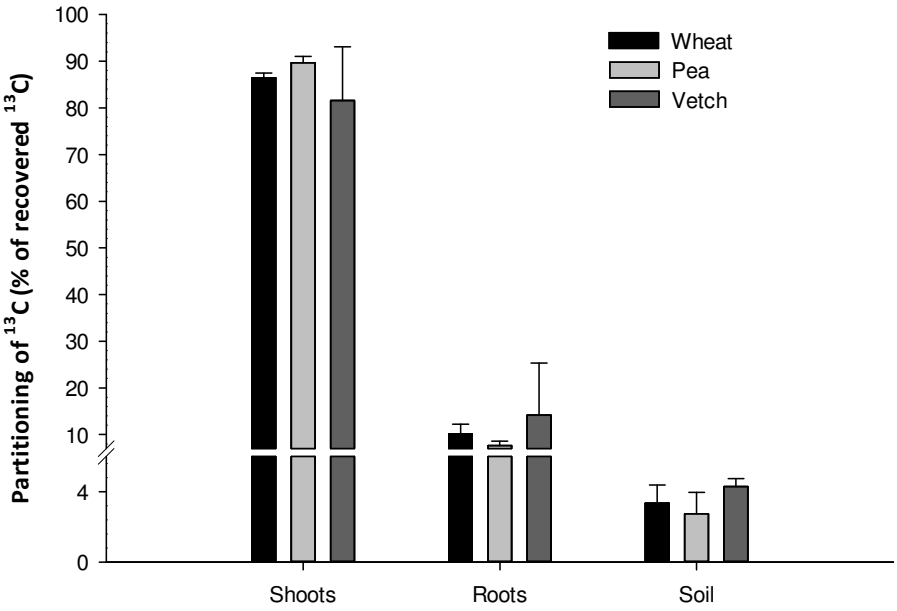

Fig. 4 Partitioning of ^{13}C in the shoots, roots and soil of three crop plants after repeat pulse labeling under field conditions. The partitioning of ^{13}C in shoots was the sum of the stems, leaves, pods and grains for wheat and pea plants and the sum of stems and leaves for vetch plants. The error bar indicates the standard error (n = 3)

3 ARTIGO II – *In situ* roots decompose faster than shoots left on the soil surface under subtropical no-till conditions[2]

3.1 Abstract

Little is known about the decomposition rates of intact roots under no-tillage (no-till) field conditions, yet better quantifying and understanding soil C dynamics under actual agricultural conditions is important for predicting impacts of land use and climate change on soil C sequestration. We aimed to estimate the actual decomposition of intact roots and shoot residues simultaneously under no-till field conditions. The experiment was conducted at Federal University of Santa Maria (UFSM), Rio Grande de Sul (RS), Brazil, in 2013-2014. Wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.) and vetch (*Vicia sativa* L.) plants were grown inside polyvinyl chloride (PVC) cylinders hydraulically forced into a field of sandy loam-textured Typic Paleudalf. At 20 days after emergence, the crops were pulse labeled weekly with $^{13}CO_2$ until the flowering stage. After harvest, the treatments were designed by combining ^{13}C-labeled shoots with unlabeled roots+soil and unlabeled shoots with ^{13}C-labeled roots+soil, resulting in six treatments (2 combinations × 3 species), plus a non-amended control treatment. Soil CO_2 emission was measured continuously by the alkaline trap method, for a total of 48 trap changes during 180 days, using closed chambers installed on the cylinders; 3 replicates per treatment were used. The amount of ^{13}C in the soil cylinders was measured after destructive sampling at day 0 and day 180. The apparent C mineralization was similar for the three species in paired treatments: 54 ± 8.8% added C for wheat, 54 ± 3.4% for pea and 51 ± 3.4% for vetch. The mineralization of roots+soil C was higher than that in the shoot residues for the three species (73 vs. 45% initial C for wheat, 76 vs. 48% for pea and 73 vs. 51% for vetch). The remaining ^{13}C in the roots and soil organic matter (SOM) on day 180 indicated both a higher rate of root-C decomposition and a higher rate of root-derived C in SOM compared to shoots. The findings emphasize that the environmental drivers of decomposition, i.e., the crop residue location and contact with soil and the soil moisture and temperature, are important factors that significantly promote root, as opposed to shoot, decomposition *in situ*, negating the consequences of their different initial chemical composition. This work provides a framework for further studies focusing on the interactions between aboveground and belowground crop residues and environmental factors under no-till field conditions.

Keywords: ^{13}C, Decomposition, Mineralization, No-till, Shoots, Soil C, Roots

[2] Article is prepared in accordance with the format of the Journal *Soil Biology and Biochemistry*.

3.2 Introduction

The better quantification of the processes of C cycling in soils and an understanding of the key drivers is required not only because of various ecosystem services associated with soil organic matter (SOM) but also because of the role of soil in C storage (Schmidt et al., 2011). Aboveground residues (leaves and stems) and belowground residues (roots and rhizodeposition) fuel decomposition, from which ultimately depends the amount of C stored in soils. Most of the studies published to date on plant litter decomposition have been focused on the decomposition of plant shoots rather than that of roots (Zhang et al., 2008). However, root characteristics and their degradation in soils has received greater attention in the past decade due to increasing recognition of their potential to enhance soil C sequestration (Puget and Drinkwater, 2001; Rasse et al., 2005; Kätterer et al., 2011; Redin et al., 2014; Menichetti et al., 2015).

The patterns of root residue decomposition and comparisons with other plant parts have largely been assessed under laboratory conditions in attempts to understand relationships between chemical and physical features and biodegradability (Jensen et al., 2005; Redin et al., 2014). Many studies have reported higher lignin contents in roots compared to shoots (e.g., Bertrand et al., 2006; Carrera et al., 2008), and this characteristic is often linked with the observed slower decay rates of roots compared to shoots (Potthast et al., 2010). However, laboratory conditions (e.g., the use of dried plant residues, reduced particle size or even grounding with incorporation into disturbed soils) do not reflect actual field conditions and thus alter the decomposition environment. Because shoots are harvested in the field and often ground and eventually reincorporated by tillage or left on the soil surface in no-till systems, the possible bias introduced by controlled conditions could affect the decomposition of roots to a greater degree than that of shoots. Furthermore, laboratory experiments do not adequately address many important factors, particularly differences in the contact between residues and soil, small-scale variability in soil conditions and climate, and the abundance and structure of soil meso-, macro- and microfauna, which can influence the decomposition process (Steffens et al., 2015). Recently, the actual importance of the chemical quality of plant residues, for roots as well as other types of plant litter, on their decomposition has been discussed, highlighting the crucial role of environmental and biotic conditions of decomposition and the need for a better consideration of the interactions between these factors (Schmidt et al., 2011; Dungait et al., 2012).

However, few studies have compared the decomposition of shoot and root residues under actual field conditions, and moreover, contradictory results have been obtained. Indeed, roots have been reported to decompose faster (Buyanovsky and Wagner, 1987; Blenis et al., 1999) or slower (Puget and Drinkwater, 2001; Williams et al., 2006) than shoot residues. Such contrasting results suggest that the different abiotic conditions experienced by surface-residues and buried/intact roots may influence their decomposition patterns, even when their chemical composition is similar. Different positions in the soil generate differences in both the physicochemical environment and the decomposer communities, which are major factors that, in addition to chemical composition, control residue decomposition (Fujii and Takeda, 2010). Decomposition of shoot and root residues under field conditions has also been studied using litter bags (Fujii and Takeda, 2010; Sanaullah et al., 2011; Freschet et al., 2013); however, the separation of roots from soil followed by washing, drying and placing inside litter bags destroys root–rhizosphere interactions, and this disruption would influence the dynamics of decay (Fisk et al., 2011). Thus, the use of the litter bag technique may misrepresent actual root decay rates (Sun et al., 2013). Root residues decompose more rapidly in soil when left undisturbed than when air-dried and mixed with moist or air-dried soil (Martin, 1989).

An important challenge under no-till field conditions is therefore understanding the interactive influence of shoot and root residues in their "natural" location without soil disturbance. Coppens et al. (2007) reported that moisture limitation was more important than chemical quality for the decomposition rate of surface-applied crop residues of contrasting quality, and Helgason et al. (2014) showed different microbial utilization of C when residues were applied to the surface or incorporated into the soil. In Brazil, for example, 27 Mha of arable lands are presently under no-till system (Boddey et al., 2010), and this is therefore a key issue. However, to our knowledge, no field study thus far has described the decomposition dynamics of shoots and intact roots in such system. Hence, the objective of this work was to study and compare the dynamics of C mineralization of shoots and intact root residues decomposing under actual field conditions of decomposition, i.e., crop shoots recycled on the soil surface and intact roots decomposing in undisturbed soil. To this end, we pulsed wheat, pea and vetch plants with ^{13}C in the field during vegetative growth and then designed "paired treatments" by combining the labeled residues with unlabeled residues ($^{13}C/^{12}C$) for the decomposition study to assess the respective contribution of shoot C and root C to the C mineralization observed and to distinguish them from native SOM mineralization. We were interested in understanding how the crop residue location, beyond differences in

chemical composition such as those observed between stems and roots, impact decomposition. An assumption was that the expected differences in decomposition between roots and shoots due to their chemical composition would be reduced under actual field conditions due to the more favorable conditions experienced by the decomposing roots.

3.3 Materials and Methods

3.3.1 Experimental site

The field experiment was performed in the experimental area of the Departments of Soils, Federal University of Santa Maria (UFSM), located in Rio Grande de Sul (RS) State, Brazil (29°41′ S, 53°48′ W; approximately 90 m elevation). The soil is classified as Typic Paleudalf according to the USDA Taxonomy (Soil Survey Staff, 2010) with 10% clay, 27% silt, 63% sand, 1.5 g.cm^{-3} bulk density and 6.6 g C kg^{-1} and -19.17 δ^{13}C in the top 0–30-cm soil layer. The experimental area has a humid subtropical climate. The annual average temperature is 19.3^{o}C, with the coldest monthly temperature below 9°C in June and the warmest above 30°C in January. The average annual precipitation is 1769 mm, without a dry season. The rainfall and air temperature during the present study period are shown in Fig 1a. Prior to the experiment, the site had been under no-tillage winter [oat (*Avena sativa*) or vetch (*Vicia sativa*)]/summer [corn (*Zea mays*) or soybean (*Glycine max*)] crop rotation for 15 yr.

3.3.2 Experimental setup and ^{13}C labeling

On 15 May 2013, an area of 25 × 25 m^2 within the experimental site was marked, cleared and fenced to study the C dynamics of wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.) and vetch (*Vicia sativa* L.) root and shoot residues. The experiment was designed according to a split-plot randomized complete block design with three replications. The main plots consisting of wheat, pea and vetch were divided into two equal microplots. One microplot in each main plot was used for pulse labeling with ^{13}C, whereas the other was un-labeled, for a total of 18 microplots. Inside each microplot, nine open-ended polyvinyl chloride (PVC) cylinders (35 cm height × 20 cm diameter, area of 0.0314 m^2) were hydraulically forced to a soil depth of 30 cm. The top of each soil cylinder was 5 cm above the soil surface with small holes on each side for water drainage. In addition, a total of twelve soil cylinders were similarly forced into soil in the area between each microplot as controls

without plants. On 29 May 2013, eight seeds of wheat, pea and vetch were hand-sown in each soil cylinder of the microplots. After emergence, the plants in each cylinder were thinned to four plants for wheat, two plants for pea and four plants for vetch. Phosphorus and potassium was applied at a rate of 50 kg ha^{-1}. Nitrogen was applied at a rate of 90 kg N ha^{-1} for wheat in the form of ammonium sulfate in six equal doses (15 kg N ha^{-1}). All other standard and cropping practices were performed during the entire experiment.

The wheat, pea and vetch plants were pulse labeled with $^{13}CO_2$ between 22 June and 18 September, 2013. The ^{13}C-labeling pulses occurred weekly for 1.5 h between 9:00 and 11:00 in the morning. The labeling was continued up to the initiation of flowering, for a total of eleven labeling pulses. To label the plants, the labeled microplots were enclosed in labeling chambers. $^{13}CO_2$ gas was generated through the reaction between 1 mol L^{-1} solution of $NaH^{13}CO_3$ (33 atom %) (Cambridge isotope laboratories, Inc, USA) with 2 mol L^{-1} HCl in a beaker placed inside the labeling chamber. The total CO_2 concentration in the labeling chamber was monitored using a portable infrared gas analyzer (IRGA). The CO_2 concentration in the chambers was maintained at approximately 266 to 400 ppm, and the $NaH^{13}CO_3$ (33 atom %) solution was added when the CO_2 levels dropped to 266 ppm during 1.5 h of labeling. Ice packs were used to minimize excessive heating and to condense excess humidity during the ^{13}C labeling. After each labeling pulse, the chambers were replaced over the labeled microplots at sunset to capture overnight $^{13}CO_2$ respiration. The chambers were removed the following morning after the CO_2 levels fell below 266 ppm. The plants were harvested on 2 October 2013 and dried to a constant weight at 40°C for 48 h in a forced-air oven.

3.3.3 ^{13}C paired treatments

We designed "paired" treatments with ^{13}C labeled and unlabeled wheat (W), pea (P) and vetch (V) shoots and roots, similar in chemical composition except for the ^{13}C enrichment (Table 1). The design consisted of two treatment combinations for each crop: ^{13}C-labeled shoots + unlabeled roots (LS) and unlabeled shoots + ^{13}C-labeled roots+soil (LR) (Fig. 2a). These treatment combinations resulted in six treatments: WLS and WLR for wheat, PLS and PLR for pea and VLS and VLR for vetch. In the WLR, PLR and VLR treatments, the soils in the cylinders were enriched with ^{13}C prior to the start of the decomposition experiment due to the plant exposure to $^{13}CO_2$ during vegetative growth. The root+soil inside the labeled and unlabeled cylinders remained undisturbed. Destructive sampling of three labeled and

unlabeled cylinders per crop was performed at the start of the decomposition experiment (day 0 = D_0) to determine the initial ^{13}C (C_0) in the soil and roots, and these values were used for subsequent calculations. The initial soil ^{13}C in the labeled cylinders corresponded to C from rhizodeposition during plant growth. Three bare soil cylinders not cropped or labeled with ^{13}C were also sampled at D_0 and used as the control for ^{13}C and soil respiration calculations.

Dried stems were cut into 10 cm pieces, and dried leaves were used intact. The "shoots" were prepared as a mixture of the stems and leaves of each species, according to their actual mean proportion, which was determined for the plants at harvest time. The shoots were placed on the soil surface inside the soil cylinders at a rate of 5 Mg ha^{-1}. The labeled shoot treatments (WLS, PLS and VLS) were prepared by placing the labeled shoots collected from the labeled cylinders of each species on the surface of the prior unlabeled soil cylinders, i.e., containing unlabeled roots+soil. Similarly, the labeled roots+soil treatments (WLR, PLR and VLR) were prepared by placing the unlabeled shoots collected from the unlabeled cylinders on the soil surface of the prior labeled cylinders, i.e., containing labeled roots+soil. For all treatments, the soil and roots remained undisturbed until the cylinders were sampled.

Soil moisture and temperature were measured by time domain reflectometry (TDR, Model: TDR 100, Campbell Equipment Inc., USA) sensors (metallic rods) and thermocouple soil temperature sensors (copper-constantan wire) in triplicate in the middle of the 0–5- and 5-15-cm soil layers inside the cylinders for each crop and control. Data loggers (Model: CS 1000, Campbell Equipment Inc., USA) were installed to record and store temperature data automatically at 30-minute intervals. Soil moisture was manually measured daily during the first week, every 2 days during the second week, every 3 days for the third week, and then every 4 or 5 days for the remainder of the experiment.

3.3.4 C-CO_2 measurements

Measurements of carbon dioxide (CO_2) emissions started on 5 October, 3 days after plant residue harvest and 1 day after the shoots were added back to the cylinders. Soil CO_2 emission was measured continuously by the alkaline trap method, for a total of 48 trap changes during the 180-day experiment (Fig. 2b, c). Closed chambers installed on the cylinders (area of 0.0314 m^2) were used for CO_2 measurements (Fig. 2d). Plastic rubbers were mounted on the upper end of the soil cylinders and lower end of the closed chambers for an air-tight seal. A plastic pot (300 mL) containing 100 mL 1 M L^{-1} NaOH was placed inside

each soil cylinder over a metal frame 5 cm above the surface residues. CO_2 measurements were performed by rotating the traps on the various cylinders of each treatment to minimize the effect of cylinder closure, particularly to protect against rain. When rain occurred during cylinders closure, an amount of water equivalent to this rain was applied to these cylinders while moving the NaOH traps. The CO_2 traps were changed at frequent intervals: daily during the first week, after 2 days during the next three weeks, after 3 to 5 days during the next 8 weeks and then weekly for the remainder of the experiment. The traps were also changed immediately after rainfall during the entire experiment. The amounts of CO_2 trapped by NaOH were measured in a 10-mL aliquot by titration with 1 M HCl after the addition of 2 mL of 1 M $BaCl_2$. The $BaCO_3$ precipitate formed was separated by vacuum filtration (glass fiber filter, porosity 1.2 μm) and dried at 65°C for 24 h. The precipitates were then weighed in a tin cap with a catalyst (PbO_2), and the isotopic ratio was measured by mass spectrometry.

3.3.5 Cylinders sampling

Three randomly selected soil cylinders from the labeled and unlabeled microplots were destructively sampled on D_0 and on day 180 (D_{180}) after collecting surface residues by excavating the cylinders to a depth of 35 cm. The soil cylinders were placed in plastic bags, transferred to the laboratory, stored at 4°C and processed within 2 days. The entire soil core (0-30 cm) was removed by cutting the soil cylinder at both sides using an electric saw. The soil core was then separated into three soil layers (0–5, 5–15 and 15–30 cm), which were weighted. All visible roots were immediately removed by hand from each soil layer; the soil of each layer was then thoroughly mixed, and 100 g moist soil was sub-sampled and suspended in 200 mL of deionized water. Fine roots were removed by gentle shaking and rinsed further with tap water until clean. The remaining soil was passed through a 2-mm sieve, air dried and systematically mixed to ensure representative sub-sampling. The soil was finely ground in a steel ball mill for the determination of total C and $\delta^{13}C$.

3.3.6 Chemical and isotopic analysis

Roots and shoots were dried to constant weight at 40°C for 48 h in a forced-air oven. One sub-sample was oven dried at 65°C for 48 h for dry matter correction, and a second sub-sample dried at 40°C was ground to 1-mm particles for chemical analysis using the methods described by Van Soest (1963); a third sub-sample was first ground in a coffee grinder and

then with a steel ball mill for C, N and $\delta^{13}C$ analysis. The total N contents of root and shoot residues were analyzed using an elemental analyzer (Flash EA 1112, Thermo Electron Corporation, Breman, Germany). The total C and $\delta^{13}C$ of the soil, carbonates and root and shoot residues were analyzed using the elemental analyzer coupled to an isotope ratio mass spectrometer (Delta V Advantage, IRMS Thermo Fisher Scientific Inc. Germany) by an interface (ConFlowIV). The characteristics of the different plant materials are shown in Table 1.

3.3.7 Calculations and statistics

The isotopic values are expressed relative to the Vienna-Pee Dee belemnite (V-PDB) reference as $\delta^{13}C$:

$$\delta^{13}C(‰) = \left(\frac{R_{sample}}{R_{V-PDB}} - 1\right) \times 1000 \quad (1)$$

$$^{13}C(\text{atom }\%) = 100 \times \frac{\left((\delta^{13}C+1000)\times R_{V-PDB}\right)}{\left((\delta^{13}C+1000)\times R_{V-PDB}+1000\right)} \quad (2)$$

$$^{13}C \text{ excess (atom }\%) = {}^{13}C \text{ sample(atom }\%) - {}^{13}C \text{ natural abundance (atom }\%) \quad (3)$$

where R_{sample} is the ratio of $^{13}C/^{12}C$ in sample and $R_{V\text{-}PDB}$ is equal to 0.011179. ^{13}C (at %) represents the percent of ^{13}C atoms of the total carbon atoms, and ^{13}C excess (at %) is the difference in the ^{13}C content between a labeled sample and a non-labeled sample (i.e., a sample at the natural abundance).

The following equation was used to estimate the amount of ^{13}C incorporated into various plant and soil pools:

$$^{13}C \text{ amount}(\text{mg m}^{-2}) = {}^{13}C \text{ excess (at }\%) \times C \text{ pool size (mg m}^{-2})/100 \quad (4)$$

where the C pool size is the carbon content of the shoots, roots and soil.

LR (roots+soil) treatments contained ^{13}C in the soil prior to the start of the experiment (D_0) due to the ^{13}C pulse labeling, which was not the case for the LS treatments. Therefore, we measured the amount of ^{13}C in the roots ($C_{0\ root}$), shoots (C_{0shoot}) and soil (C_{0soil}) in both the labeled and unlabeled microplots to calculate the total ^{13}C ($C_{0\ total}$).

$C_{0\ total} = C_{0\ root} + C_{0\ shoot} + C_{0\ soil}$

C_{0total} for wheat, pea and vetch was 395, 264 and 418 mg ^{13}C m^{-2} in the LR (roots+soil) treatments and 997, 959 and 727 mg ^{13}C m^{-2} in the LS treatments, respectively. At the end of the experiment, the ^{13}C remaining (^{13}C balance) in the cylinders (C_{180}) was calculated in similar way, except that the cumulative $^{13}CO_2$ evolved was also included in the total:

$C_{180\ total} = C_{180\ root} + C_{180\ shoot} + C_{180\ soil} + C_{180\ CO2}$

Because three sources of CO_2 were involved, we used a mass balance approach to calculate the contribution of each source to total soil respiration for each crop species, as described by Puget and Drinkwater (2001). We also assumed that the decomposition of root and shoot residues was identical in both the paired treatments, regardless of the $\delta^{13}C$ signature.

$$\delta^{13}C_{LR-CO_2} = x(\delta^{13}C_{LR}) + y(\delta^{13}C_{US}) + (1-x-y)\delta^{13}C_{N-OM} \quad (5)$$

$$\delta^{13}C_{LS-CO_2} = x(\delta^{13}C_{UR}) + y(\delta^{13}C_{LS}) + (1-x-y)\delta^{13}C_{N-OM} \quad (6)$$

$\delta^{13}C_{LR\text{-}CO2}$ represents CO_2 $\delta^{13}C$ respired from the LR (roots+soil) treatments, $\delta^{13}C_{LR}$ represents the initial $\delta^{13}C$ of the labeled roots, $\delta^{13}C_{US}$ represents the initial $\delta^{13}C$ of the unlabeled shoots, $\delta^{13}C_{LS\text{-}CO2}$ represents CO_2 $\delta^{13}C$ respired from the LS treatments, $\delta^{13}C_{UR}$ represents the initial $\delta^{13}C$ of the unlabeled roots, $\delta^{13}C_{LS}$ represents the initial $\delta^{13}C$ of the labeled shoots, and $\delta^{13}C_{N\text{-}om}$ represents the initial $\delta^{13}C$ of the control soil SOM. *x* represents the proportion of CO_2 respired from roots+soil, *y* represents the proportion of CO_2 respired from shoot residues, and $1-x-y$ represents the proportion of CO_2 respired from native SOM.

We solved *x*, *y* and $1-x-y$ by using equations (5) and (6), and the actual C mineralization from LR (roots+soil), LS and SOM was calculated by multiplying *x*, *y* and $1-x-y$ with the amount of C-CO_2 evolved at each sampling date in relation to $C_{0\ total}$.

All results are shown as the means of three replicates. ANOVA was used to compare apparent and actual mineralization rates, cumulative C mineralization and shoot and root $\delta^{13}C$ values. The data were analyzed as a split-plot randomized complete block design with the crop (C) as the main plot and the source of ^{13}C (T) (i.e., root or shoot residue, C source) as the sub-plot, and the plot was induced as a random factor (Table 2). Differences between means were calculated based on the least significant difference (LSD) test ($P < 0.05$).

3.4 Results

3.4.1 Climatic conditions during the experiment

Southern Brazil has a humid subtropical climate associated with high summer temperatures and heavy rainfall; over 60% of the annual rainfall occurs during summer months. Although the monthly distribution of rainfall is quite uniform, day-to-day variability is high and occasionally, rainfall exceeds 100 mm day^{-1}. The cumulative rainfall during the decomposition experiment was 1015 mm, and the mean daily air temperature remained > 20°C between days 45 and 150 (Fig. 1a). During the first 45 days, the soil volumetric moisture content varied between 0.13 and 0.23 g H_2O 100 g^{-1} soil in the 0–5-cm soil layer and between 0.19 to 0.24 g H_2O 100 g^{-1} soil in the 5–15-cm soil layer (Fig 1b). The moisture content decreased in both soil layers during the period of higher air and soil temperature (between 80 and 145 days) compared to the peak values (between 40 and 65 days), and the soil moisture content at 5 to 15 cm remained significantly higher than that at 0 to 5 cm during this time. The soil temperatures in the 0–5- and 5–15-cm layers ranged between 20 to 28°C during the first 45 days and then were > 30°C between 75 and 90 days (Fig. 1c).

3.4.2 Total C mineralization

The total C-CO_2 evolved did not differ significantly between the paired treatments for wheat (WLS vs. WLR), pea (PLS vs. PLR) and vetch (VLS vs. VLR) at any time during 180 days of decomposition (Table 2). Therefore the C-CO_2 values obtained with the paired treatments for each crop were averaged to present the C mineralization rates and cumulative C mineralization over time and to compare them among the crop species (Fig. 3a, b). The pattern of C mineralization rates were fairly similar between the species, marked by sharp fluctuations during the first 20 days, followed by a gradual decrease from day 45 and decreasing more gradually until the end of the experiment. The initial fluctuations in mineralization rates appeared to be affected by rain events. The maximum daily C mineralization rates were observed during the first 2 to 9 days, peaking at 86.0 ± 2.5 kg C-CO_2 ha^{-1} day^{-1} for vetch, at 70.6 ± 3.2 kg C-CO_2 ha^{-1} day^{-1} for pea and at 62.6 ± 2.4 kg C-CO_2 ha^{-1} day^{-1} for wheat. The next peak values were observed during days 17 to 19, soon after rainfall (60 mm), and on day 40, two days after a rain event of 133 mm (Fig. 1a). Overall, the wheat and pea treatments showed similar mineralization kinetics after 60 days, and their C

mineralization rates remained higher than those of vetch, though not significantly different for most of the time. As expected, low mineralization rates (19.8 ± 5.2 kg C-CO_2 ha^{-1} day^{-1}) occurred for SOM in the control soil (un-amended), decreasing with time throughout the incubation period. However, peak values were also observed for the control soil on day 17 (27.1 ± 3.8 kg C-CO_2 ha^{-1} day^{-1}) and day 40 (36.7 ± 2.4 kg C-CO_2 ha^{-1} day^{-1}) after rainfall episodes.

As expected, total cumulative mineralization was significantly higher ($P < 0.05$) in the residue-amended treatments (WLS and WLR, PLS and PLR, VLS and VLR) compared to the control soil (Fig. 3b). Expressed as a function of the initial shoot-C + (roots+soil)-C, apparent C mineralization was 54 ± 8.8% for wheat, 54 ± 3.4% for pea and 51 ± 3.4% for vetch residues, assuming that the mineralization of SOM was equivalent in both residue-amended and non-amended soils.

3.4.3 Contribution of C sources to total soil respiration

The use of labeled "paired" treatments allowed for calculating the partitioning of the total respired C-CO_2 into C derived from labeled shoots, C derived from labeled roots+soil and C derived from SOM. First, the decomposition of labeled shoots in the presence of unlabeled roots+soil (WLS, PLS, VLS) and unlabeled shoots in the presence of labeled roots+soil (WLR, PLR, VLR) was clearly reflected in the $\delta^{13}C$ values of total soil-respired CO_2 (Fig 4a, b, c). The $\delta^{13}C$ values of the LS and LR treatments showed an opposite trend initially, whereby LS $\delta^{13}C$ was very low initially and increased quickly and strongly and the $\delta^{13}C$ values of LR were quite high initially and decreased quickly and concomitantly. The $\delta^{13}C$ signals of both LS and LR then decreased continuously over time until the end of incubation. The significantly higher $\delta^{13}C$ values of C-CO_2 evolved from the LS compared to the LR treatments were due to higher amounts of shoot biomass compared to root biomass decomposing in the soils, inducing a lower dilution of the labeled C-CO_2 evolved from the labeled shoots treatments compared to the labeled roots+soil treatments.

The $\delta^{13}C$ values of the C-CO_2 evolved from the LS treatments notably varied over time when compared to those from the LR treatments. This suggests a greater dependence of the mineralization of shoots compared to roots on the observed fluctuations of moisture (Fig. 1a). This was clearly reflected at day 83, when the $\delta^{13}C$ values of the labeled shoots dropped to those of the labeled roots (Fig. 4a, b, c) due to a short dry spell (Fig. 1a). The contributions of each source (shoots, roots+soil and native SOM) to total soil respiration over time deduced

from these $\delta^{13}C$ values are given in Fig. 4d, e, f, for wheat, pea and vetch, respectively. Overall, the patterns of mineralization were similar for the three crops, with a contribution of belowground residues (roots+soil) ranging from 10 to 20% of the total C-CO_2 evolved, which represented a larger, more variable and decreasing contribution by the shoots over time. For the three crops, the native SOM was the major CO_2 source, exhibiting a decrease during the first 30 days (when the shoot contribution was high) and then increasing again until the end of experiment.

3.4.4 Actual mineralization of residues-C using ^{13}C

The actual rates of C mineralization of the shoots and roots+soil (Fig. 5a, b, c) were calculated from the total C-CO_2 evolved and from the proportion of the different sources. The actual rates of LS and LR C mineralization showed similar patterns for three crop species, i.e., initial higher roots+soil mineralization rates and lower rates of shoot mineralization. The mineralization rates of the shoots and roots+soil of vetch residues were higher compared to pea and wheat, mainly during the first 15 days. Significant differences between LS and LR were observed during the course of decomposition and were more frequent for pea compared to wheat and vetch. The C mineralization rates of the LS and LR residues decreased gradually, and the average rates after the first 30 days were less than 1% of the added ^{13}C day^{-1}.

Cumulative actual residue-C mineralization showed that the C mineralization of roots+soil was much higher than that of shoots for the three crop species (Fig. 6). The dynamics of mineralization showed differences among the shoots; in particular, a faster decomposition for vetch compared to pea and wheat shoots was significantly different during the first 37 days, with VLS> PLS> WLS. By the end of the decomposition period, the actual amount of C mineralized was 45.5 ± 4.2% added C for wheat (WLS), 48.9 ± 2.9% added C for pea (PLS) and 51.1 ± 1.6% added C for vetch (VLS). Cumulative C mineralization of roots+soil also varied significantly over time among the crop species, with a much slower mineralization rate in the wheat treatment. However, at day 180, no significant differences in C mineralization were found among the three crop species, with 76.8 ± 2.6% initial ^{13}C for pea (PLR), 73.5 ± 13.6% for wheat (WLR) and 70.8 ± 13.2% for vetch (VLR).

3.4.5 Native SOM mineralization

The use of $\delta^{13}C$ values also allowed us to estimate the native SOM-derived C-CO_2 for the treatments amended with crop residues and to compare these values with the C-CO_2 emitted from the control soil (without residues) (Fig. 7). SOM mineralization was greater in the residue-amended treatments than in the control treatment during the early period of decomposition (0–45 days), with a 24% increase in C-CO_2 evolved compared to the control treatment during the first 12 days. No significant differences were found after day 60 and until the end of field decomposition. Cumulative SOM mineralization in the residues treatments was 1026 ± 72 kg C ha^{-1} for wheat, 1025 ± 25 kg C ha^{-1} for pea and 979 ± 31 C kg ha^{-1} for vetch compared to 951 ± 15 kg C ha^{-1} for the control soil. This additional mineralization was therefore 74 ± 40 kg C ha^{-1} for wheat and pea and 28 ± 38 kg C ha^{-1} for vetch residue compared to the control soil.

3.4.6 ^{13}C balance

The ^{13}C balance was calculated between day 0 and day 180 for the LS and LR treatments (Table 3) and was 84.0 ± 4.2% initial ^{13}C for wheat, 85.8 ± 5.5% for pea and 83.3 ± 1.0% for vetch. In contrast, the RL treatments exhibited higher ^{13}C recovery than the LS treatments, ranging from 101.1 ± 5.6% for pea to 104.9 ± 8.8% for wheat roots. The ^{13}C content of the soil, after removing the labeled root or shoot particles, did not change much over time: it was very small in the LS treatments, which is consistent with the limited contact between decomposing shoot residues and soil, and much higher but rather constant between day 0 and day 180 in the LR treatments, which suggests that some ^{13}C fueled this pool during root decomposition concomitantly with ^{13}C losses due to mineralization. At the end of the experiment, the proportion of remaining ^{13}C in this fraction was much greater in the LR treatments, as expected.

The recovery of ^{13}C in the remaining shoot C fraction was much greater compared to the remaining root C fraction for the three species, but the variation between day 0 and day 180 was within the same range: -70 to -74% of the initial ^{13}C for roots vs. -64 to -72% initial ^{13}C for shoots.

3.5 Discussion

3.5.1 Paired treatments and C mineralization

Our study was designed to investigate the short-term C mineralization dynamics of the shoot residues and intact roots of three winter crops under no-till field conditions. The use of "paired" treatments (^{13}C labeled shoot + unlabeled roots+soil and unlabeled shoot + ^{13}C labeled roots+soil) was implemented to estimate the actual contributions of SOM and shoot- and root residue-derived CO_2 decomposing simultaneously to total soil respiration and to compare shoot and root mineralization for each species. Accordingly, it was crucial to use labeled and unlabeled crop residues and soil cylinders that were similar in their C content and chemical composition, except for the labeling. The comparison of the total C-CO_2 evolved between paired cylinders for each species showed similar kinetics and rates of C mineralization between the two treatment combinations, indicating that the *in situ*-labeling of plants prior to the decomposition study did not affect the subsequent C mineralization processes, which was expected from the initial quantification and characterization of the plant litter (shoots and roots). There are few studies in literature in which "paired" treatments have been used to study the C dynamics of shoot and root residues (e.g., Gale and Cambardella, 2000; Puget and Drinkwater, 2001). Similar C mineralization between paired cylinders was an absolute prerequisite for applying the dilution equations proposed by Puget and Drinkwater (2001) when estimating the amount of C mineralizing from each source (i.e., SOM, shoots, roots+soil).

3.5.2 ^{13}C balance

The ^{13}C balance established for the various measured pools provided two patterns according to the source of the labeling. The lack of recovery of ^{13}C from LS treatments, equivalent to approximately 15% of the initial added C, could be attributed to the underestimation of ^{13}C-CO_2 evolved. Indeed, a lack of recovery of ^{13}C is usually obtained in studies involving labeled C, which can be attributed to losses in C-CO_2 trapping during mineralization (Puget and Drinkwater, 2001). Puget and Drinkwater (2001) reported that the static chamber technique overestimates CO_2 when fluxes are low and underestimates it when fluxes are higher than 0.24 g m^{-2} $hour^{-1}$. These authors also suggested the use of the static chamber technique for C mineralization dynamics but did not use such data for calculating the

C balance. Similarly, Gale and Cambardella (2000) recovered less than 100% of applied C after 180 days from shoot and root residues under laboratory conditions. As loss of ^{13}C-CO_2 could occur when opening the cylinder cover to change the NaOH trap, the manual application of water after removing the cover to compensate for rain occurring when the cylinders are closed could lead to extra ^{13}C-CO_2 emissions, particularly with shoot mulches at the soil surface, which are not captured by the NaOH trap. The peaks of $^{13}CO_2$ emissions derived from shoot-C, observed especially after rainfall events, support this hypothesis.

In contrast, this was not the case for the LR (roots+soil) treatments, having a full ^{13}C balance, which was accompanied by high ^{13}C recovery as C-CO_2. The fluxes of ^{13}C in these treatments were lower but more regular, which could explain the reduced influence on ^{13}C losses of soil rewetting by rain. We also cannot exclude the possibility of $^{13}CO_2$ emissions due to the mineralization of roots+soil below the 30-cm soil depth i.e., outside the volume of the soil sampled in the cylinders. When removing the initial cylinders, some roots were observed below 30 cm depth at day 0, but it was impossible to quantitatively remove these roots. Therefore, we acknowledge the possibility of an overestimation of root C mineralization (i.e., underestimation of the initial source of soil (roots + rhizodeposition ^{13}C). The full ^{13}C balance for the LR (roots+soil) treatments could result from the combination of lower ^{13}C-CO_2 losses than for the LS treatments, with an extra contribution of ^{13}C-CO_2 evolving from the deeper soil layer.

3.5.3 Shoots and roots mineralization

In addition to aiding in the understanding of decomposition under real field conditions, *in situ* pulse-labeling with ^{13}C during plant growth and the use of undisturbed soil cylinders to study decomposition induced a disadvantage of labeling, the C derived from rhizodeposition in addition to that from roots. The labeled plant-derived ^{13}C in the soil fraction in the LR (roots+soil) treatments at D_0 represented approximately 25% of the total initial ^{13}C in the soil. Therefore, a significant contribution of this labeled pool to the evolved ^{13}C-CO_2 in the LR (roots+soil) treatments cannot be excluded but could not be quantified separately. This suggests that the mineralization rate of the "root" ^{13}C could be overestimated by including the initial plant-derived ^{13}C in the soil. Assuming that the turnover of this fraction would be faster than the degradation of the roots (Lu et al., 2003), the initial rates of root mineralization could be overestimated. However, the high root C mineralization was also confirmed by the low remaining root C pool after day 180. Indeed, the decrease in shoot and

root ^{13}C during the incubation was slightly in favor of roots, with -70 to -74% initial ^{13}C for roots vs. -64 to -72% initial ^{13}C for shoots. The smallest difference between shoots and roots was obtained with the vetch residues and the largest difference with the wheat residues. This result appears to contradict most previous studies, under either field (Puget and Drinkwater 2001; Williams et al., 2006) or laboratory conditions (Gale and Cambardella, 2000; Lu et al., 2003; Abiven et al., 2005), showing much lower decomposition or mineralization of root-C compared to the aboveground parts of a given crop and a higher contribution of roots to stabilizing soil C (Rasse et al., 2005; Kätterer et al., 2011). This pattern for leaf and stem vs. root decomposition was attributed to a higher intrinsic chemical recalcitrance to decomposition of roots compared to shoots (Rasse et al., 2005; Bertrand et al., 2006; Yanni et al., 2011), which appears to be quite universal (Freschet et al., 2013). In addition, differences in functional traits between roots and shoots were also confirmed in this study based on the chemical composition of roots from wheat, vetch and pea extracted from soils at the start of the experiment. However, Buyanovsky and Wagner (1987) found increased mineralization of intact roots (53% of added C) compared to surface shoot residues (49% of added C) after 1 year.

Therefore, we believe that the difference between the results obtained in this study and most of the results in the literature can be explained by the fact that in most previous studies, the conditions of decomposition did not represent actual field conditions of root decomposition *in situ*, i.e., roots were disturbed either by collection from soil and incorporated back into soil by plowing (e.g., Puget and Drinkwater, 2001; Williams et al., 2006) or by burying litter bags containing roots (e.g., Saunallah et al., 2011). In these studies, shoot residues were incorporated into the soil, which also favors rapid mineralization compared to no-till conditions of surface decomposition. This means that in addition to the chemical composition (relative concentration in SOL and LIG), differences in shoot and root locations greatly influence decomposition rates. Previous works have indicated that decomposition rates for many different crop residue types are consistently higher for residues that are buried compared to those placed on the soil surface (Summerell and Burgess, 1989; Soon and Arshad, 2002; Coppens et al., 2006). The belowground environment is considered to be favorable for decomposition in terms of moisture, temperature, availability of exogenous nutrients, particularly N (Berg and McClaugherty, 2003), and the activity and composition of decomposer populations (Osono et al., 2006). As the roots were intact and the root-rhizosphere interaction was not disturbed, the kinetics of CO_2 fluxes due to root residue decomposition in our field experiment was governed by the combined influence of the

location, soil moisture and temperature. A sufficient soil moisture content with favorable soil temperature (25°C at the depth of 15 cm) generated ideal conditions for heterotrophic activity and root mineralization in our experiment during field decomposition. Another factor that may have influenced the higher root C mineralization was the intimacy of contact between the soil and roots. Using ^{13}C-labeled barley residues, Helgason et al. (2014) showed that the retention of ^{13}C by soil biomass was approximately 50% lower when the barley residues were left on the soil surface compared to when the same residues were incorporated into the soil by tillage, and this was attributed to greater soil-residue contact, enhancing residue C availability. The no-till approach minimizes the physical disturbance of the soil and for surface-applied residues, limits the access of decomposers to fresh residues. In the present study, because the roots were intact and surrounded by soil particles, C mineralization was most likely increased due to the increased area of contact. This was evidenced by the rather stable ^{13}C-CO_2 fluxes from the LR (roots+soil) treatments compared to the LS treatments, with the later clearly being affected by fluctuations in the moisture content at the soil surface associated with rainfall. This was also shown by the high ^{13}C labeling of the CO_2 emitted from the LR (roots+soil) treatments from day 1; in contrast, the labeling of C-CO_2 from the LS treatments was initially very low and increased over time. Furthermore, the root diameter influences the decomposition and turnover of roots, with faster decomposition and turnover for small fine roots (< 2 mm diameter) (Pacaldo et al., 2014). Although we did not measure root diameters in the present work, Redin et al. (2014) reported 100% fine roots for wheat, pea and vetch grown under similar conditions. According to Sun et al. (2013) undisturbed root-rhizosphere interactions influence root decomposition, especially for fine roots. Conversely, the shoots of wheat, pea and vetch, composed of a mixture of stems and leaves, have coarser particle sizes than their roots.

It is notable however that the remaining ^{13}C in the SOM was much higher in the labeled roots+soil treatments than in the labeled shoot treatments. If we consider that part of this fraction at day 180 was derived from decomposing roots, in the long term, roots would contribute more to soil C storage than shoots decomposing at the soil surface. This higher input to SOM may result from higher physico-chemical interactions with soil particles (Schmidt et al., 2011). We think that there is no contradiction between the high and rapid decomposition kinetics of roots compared to shoots due to more favorable conditions for decomposition in the short term and increased stabilization of the carbon derived from roots + rhizodeposition in the long term because of the intimate contact with the soil and possibly the chemical nature of roots. The analysis of shoot- and root-derived C in the long term should

resolve this question. These results confirm that the importance of the chemical nature of plant litter on its decomposition depends on the accessibility of the substrate to decomposers and on environmental factors influencing decomposition (Dungait et al., 2012).

The kinetics of the CO_2 fluxes from the shoot residues (both total C-CO_2 and the $\delta^{13}C$ signal of the respired CO_2) clearly responded to the rainfall events, with the peak values after each rainfall attributed to enhanced microbial activity by the rewetting of the residues and the rapid decrease in activity between rainfall events due to the loss of residue moisture due to the high air temperatures in summer. During our study, heavy erratic rains occurred, which resulted in the rapid wetting and drying of the shoot residues, limiting the amount of time during which the conditions were favorable for decomposers. Iqbal et al. (2015) evaluated the effect of rainfall regimes under laboratory-simulated rain pulses and concluded that surface mulch with light and frequent rainfall remained 2–3 times wetter compared to the same mulch under heavy infrequent rainfall. The water content of residue has a positive effect on decomposition rates (Berg and McClaugherty, 2003) due to the promotion of decomposer activity (Osono et al., 2003). Lee et al. (2014) evaluated the effects of crop residue location with simulated rainfall pulses and also reported a strong interaction between the frequency of rain pulses and the placement of crop residues (in mulch at the surface vs. buried in the soil). Another explanation for the reduced decomposition rates of shoot residues may be limited contact with the soil. Coppens et al. (2006) estimated that only 10% of oilseed rape residue left at the soil surface was in direct contact with the soil, which was not the case for roots.

3.5.4 Effects of residue quality

Overall, there was little effect of the type of crops on the total C mineralized in 180 days, even though the vetch-amended plots initially mineralized faster. This result can be explained by the difference in the intrinsic decomposability of residues, with vetch shoots having higher N and higher SOL contents than the two other species. However, the close pattern in total C-CO_2 evolved in treatments among the three species also resulted from the fact that most of the evolved C-CO_2 derived from the SOM source and was not affected by the type of crop residue added. For shoot- and roots+soil-derived C-CO_2 kinetics, the “classical” two phases of mineralization were observed: an initial phase of rapid mineralization followed by a phase of slower mineralization. The faster phase was due to the decomposition of easily degradable C present in shoot and root residues, as shown by the Van Soest analysis, with a soluble pool representing 251 to 541 g kg^{-1} residue; this pattern is consistent with most

previous studies on residue decomposition (e.g., Puget and Drinkwater, 2001; Williams et al., 2006; Abiven et al., 2005, Redin et al., 2014). However, C mineralization in the wheat and pea treatments was not notably different, despite the fact that the pea residues had higher N contents and higher SOL contents than the wheat residues. Indeed, we hypothesize that the lower LIG contents and higher CEL and HEM contents in the wheat residues compared to the pea residues counter-balanced the low SOL content. Our results are consistent with several residue decomposition studies showing that rapid decomposition rates for high-quality litter are typically not sustained due to exhaustion of the easily degradable C; during later stages of litter decay, decomposition rates for high-quality litter slow more rapidly than do rates for low-quality litter (Cotrufo et al., 2013).

3.5.5 Apparent and actual C mineralization

The use of labeled ^{13}C plant residues allowed calculation of the actual residue-derived C mineralized, i.e., avoiding the calculation of apparent C mineralization using the difference in C mineralization compared to a control (non-amended soil). Many studies have shown that the actual mineralization of plant residues is usually lower than the apparent mineralization due to differences in SOM C mineralization between amended and non-amended soils (e.g., Bastida et al., 2013; Aita et al., 2012). In the present study, higher C-CO_2 emissions from native SOM in the residue-amended treatments compared to the control soil were clearly observed during the first two weeks of decomposition, with approximately 74 ± 40.4 kg (wheat and pea) and 28 ± 37.9 kg (vetch) extra soil C ha^{-1} emitted as CO_2, which corresponded to approximately 0.23% (wheat and pea) and 0.09% (vetch) of total soil C in the top 30 cm of the soil. However, the cumulative amount of C mineralized from native SOM with or without residue addition was not significantly different after 180 days. This extra mineralization of native SOM in the early stage of decomposition could result from two processes. First, the acceleration of native soil organic C (SOC) decomposition could be due to a priming effect, i.e., the supply of fresh organic C to soil microorganisms increased the degradation of native SOM by these soil microorganisms (Nottingham et al., 2009; Garcia-Pausas and Paterson, 2011; Guenet et al., 2012). Second, the additional mineralization could also result from more favorable soil conditions for SOM mineralization in the residue-amended cylinders compared to the control cylinders. Indeed, in our study, the control treatment was a bare surface (without any residue cover), and we measured a small difference in soil water content (0.04% higher) in the residue-amended soil compared to the bare soil.

Increased mineralization of native SOM in the upper soil layers due to the higher water content of mulch-covered soil compared to bare soil was also observed by Coppens et al. (2007) in a column experiment. Additionally, the comparatively higher soil temperature of the control soil (+ 0.5^{o}C higher on an average during entire experiment than the residue-amended soil) might reduce SOM mineralization in the control soil (Bontti et al., 2009). The two processes are not mutually exclusive; however, considering that the main difference occurred shortly after the start of plant residues decomposition, we hypothesized that the additional C mineralization could be due to a real priming effect.

3.6 Conclusions

This study showed that in agricultural no-till systems under subtropical conditions, the decomposition of intact roots belowground was rapid during the first 6 months, greater than that of shoot residues decomposing aboveground. However, in the short term, the proportion of C derived from plant residues remaining in the SOM was higher for roots than for shoots, which was attributed to the more intimate contact of the decomposing roots with the soil. In addition, the environmental conditions of root and stem decomposition masked the differences linked to the chemical attributes of the roots and stems, which are generally observed under controlled conditions i.e., equivalent conditions of decomposition for shoots and roots. These findings highlight the importance of our study in revealing the role of a realistic experimental system (actual no-till field conditions) on residue decomposition, especially for intact roots, whereas high lignin contents are often used as justification for the slow decomposition of roots in laboratory experiments. The findings do not suggest that plant litter traits and, in particular, the chemical composition of litters are not important for decomposition rates but rather that their influence depends on other environmental factors. Under no-till conditions, this study also reveals the importance of climatic conditions, such as those encountered in subtropical regions during summer months after winter crop harvests, with strong but erratic rainfall combined with high temperature, in greatly influencing the dynamics of residue mulch mineralization, which is primarily controlled by mulch drying/wetting sequences. Studies of the fate of shoot- and root-derived C in soil in the long term are required to better understand the contribution of shoots and roots to soil organic matter stabilization.

Acknowledgements

The first author's doctoral study was funded by a CNPq-TWAS postgraduate fellowship program. This work was supported by the Brazilian government through the Conselho Nacional de Desenvolvimento Científico e Tecnologico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS). The bilateral Brazilian and French collaboration was funded under Program CNPq—Ciência sem Fronteiras Process Number 401724/2012-3. CN-MIP project (ANR-13-JFAC-0001) provided a grant to M.M. Tahir for his leave at INRA, Reims, France. We thank Prof. Dr. Paulo Ivonir Gubiani for his help with equations and André Friderichs for the ^{13}C isotopic analysis. We thank Dr Denis Angers from AAC for his insightful comments on a previous version of this work.

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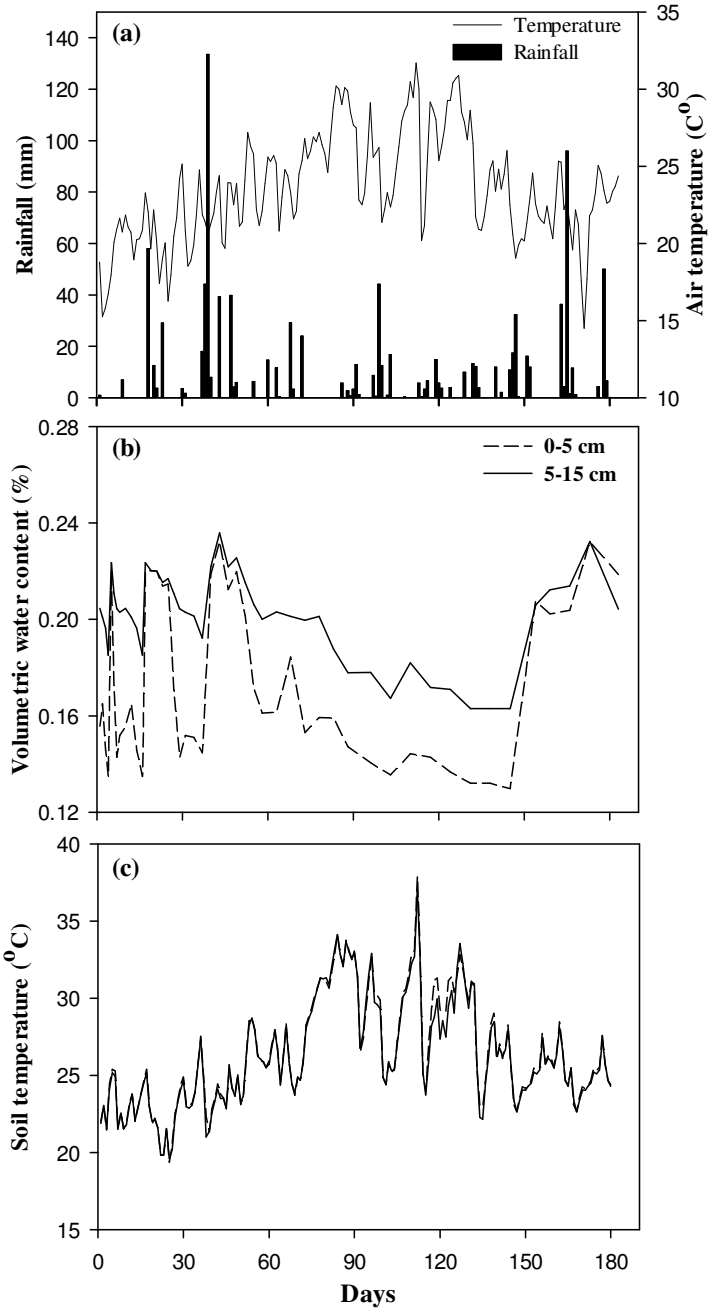

Fig. 1 Daily rainfall and mean air temperature (a) temporal dynamics of the volumetric water content (b) and soil temperature (c) of two soil layers (0-5 and 5-15cm) during the decomposition of shoot and root residues under no-tilled field conditions at Santa Maria, Rio Grande do Sul, Brazil. Daily mean soil temperature values represent averages of measurements from three field replicates made every 30 mins. Day 0 is the start of the field decomposition study (04-10-2013).

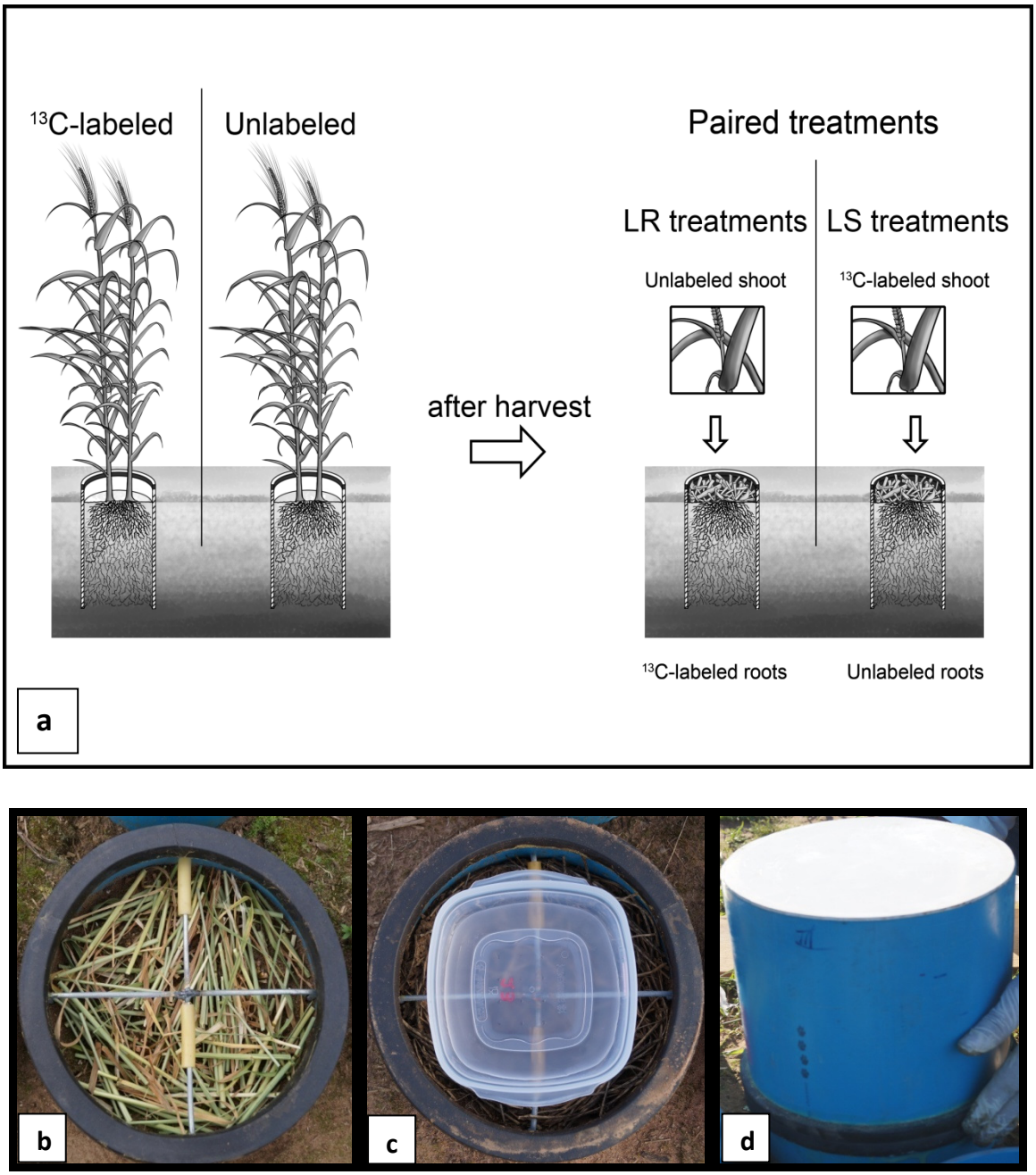

Fig. 2 Diagrammatic illustration of "paired treatments" (a) and photographic presentation of surface residues (b), sodium hydroxide trap (c), and static chamber (d) for measuring C-CO_2 emissions under field conditions.

Table 1

Amounts of Dry matter (DM), Carbon (C) and Nitrogen (N) added, initial chemical composition of the shoots and roots and $\delta^{13}C$ of shoots, roots residues and soil after pulse labeling with ^{13}C under field conditions.

			Residues										**Soil (0 -30 cm)**		
Crops	**Treatments**	**Combination**	DM	C	N	C/N	$\delta^{13}C$	SOL	CEL	HEM	LIG	$^{b13}C_{residues}$	$\delta^{13}C$-D_0	$^{c13}C_{rhizo}$	$^{d}C_{soil}$
			kg ha^{-1}				‰	g kg^{-1}				mg	‰	mg	kg ha^{-1}
Wheat	WSL[a]	shoot labeled	5088	2209	74	30	383	377	298	287	38	31.3	–	–	–
		root unlabeled	1298	522	19	27	-28	251	360	301	88	–	-19.33	–	166
	WRL	shoot unlabeled	5083	2222	72	31	-28	385	292	278	45	–	–	–	–
		root labeled	1341	536	20	27	473	260	352	296	92	9.5	-16.73	2.9	166
Pea	PSL	shoot labeled	5065	2228	94	24	365	394	363	156	87	30.1	–	–	–
		root unlabeled	789	342	26	13	-30	342	275	240	143	–	-19.28	–	148
	PRL	shoot unlabeled	5059	2197	89	25	419	406	348	155	91	–	–	–	–
		root labeled	941	395	29	14	-30	346	272	251	130	6.0	-17.15	2.2	148
Vetch	VSL	shoot labeled	5081	2165	169	13	278	534	268	133	66	22.9	–	–	–
		root unlabeled	2089	819	65	13	-30	321	286	268	125	–	-19.48	–	240
	VRL	shoot unlabeled	5082	2175	158	14	-31	541	262	129	68	–	–	–	–
		root labeled	1796	717	55	13	330	326	297	258	120	10.0	-16.41	3.1	240

[a]WSL=wheat shoot labeled + wheat root unlabeled: WRL= wheat shoot unlabeled + wheat root labeled + soil: PSL pea shoot labeled + pea root unlabeled: PRL pea shoot unlabeled + pea root labeled + soil: VSL vetch shoot labeled + vetch root unlabeled: VRL= vetch shoot unlabeled + vetch root labeled + soil. ^{b13}Cresidues: amount of ^{13}C present in labeled roots and shoots on day 0.

^{c13}Crhizo: amount of ^{13}C present in labeled roots treatments in the form of rhizodeposits per cylinder on day 0.

dC_{soil}: amount of C by rhizodeposition in soil prior to decomposition experiment, estimated after plant harvest on day 0 and assumed to be same in both labeled microplots and unlabeled microplots. Values are the mean of 3 replicates.

Table 2

Analysis of variance to investigate the interactive effects of crops (C), Carbon source (T) and Time (D) on selected variables.

	Crops[a] (C)	C source[b](T)	Time (D)	C × T	C × D	D × T	C × T × D	CV[c]
Total C mineralization rate ($kg\ C\ ha^{-1}\ d^{-1}$)	***	ns	***	ns	***	ns	ns	10.21
Apparent cumulative mineralization ($kg\ C\ ha^{-1}$)	***	ns	***	ns	***	ns	ns	4.52
$\delta^{13}C$ (‰)	***	***	***	***	***	***	***	29.98
Actual mineralization rate ($kg\ C\ ha^{-1}\ d^{-1}$)	***	***	***	***	***	***	***	26.88
Actualcumulativemineralization ($kg\ C\ ha^{-1}$)	***	***	***	***	ns	***	ns	12.90
SOM mineralization ($kg\ C\ ha^{-1}\ d^{-1}$)	***	nr	***	nr	***	nr	nr	16.75

[a]wheat, pea and vetch.
[b]shoot labeled or root labeled.
[c]coefficient of variation (%)*** $P < 0.001$. ns= not significant. nr=not relevant.

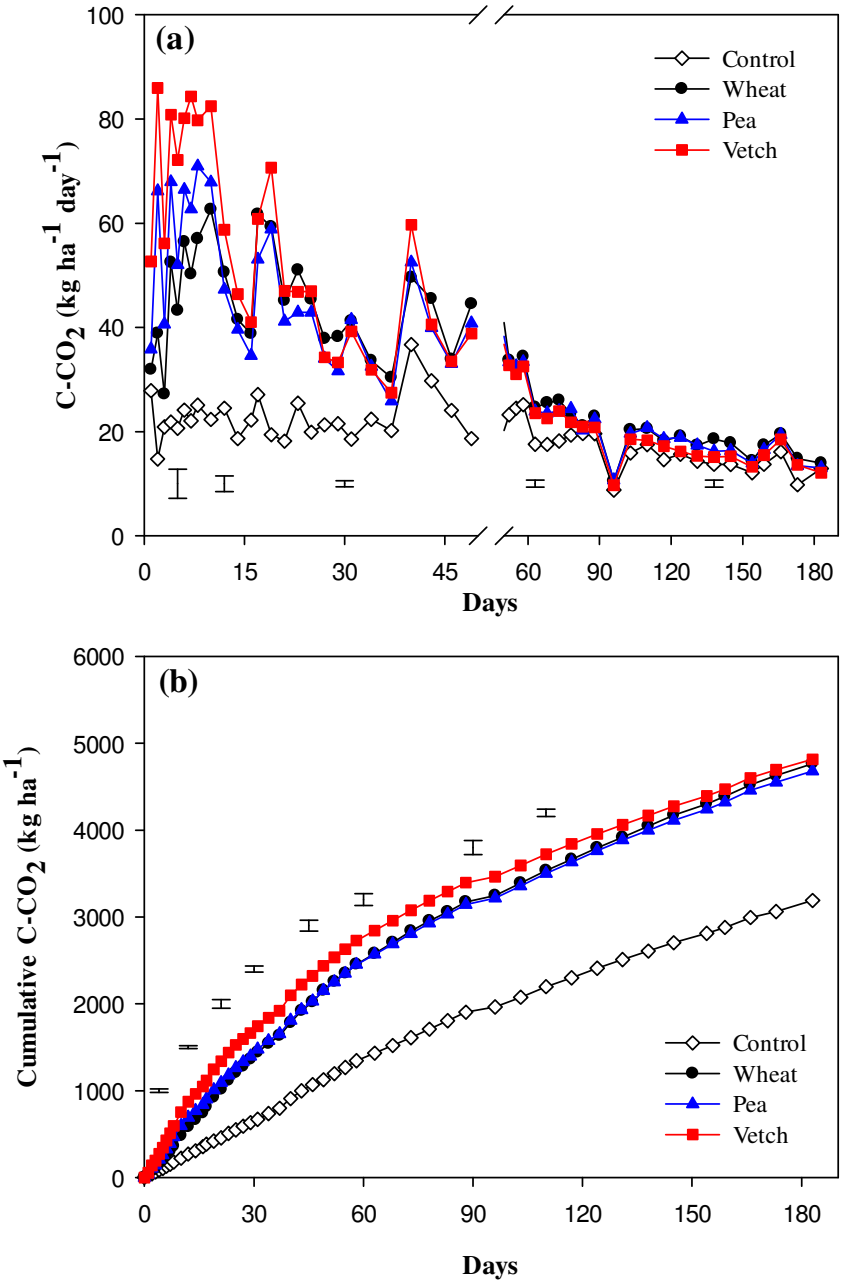

Fig. 3. Apparent C mineralization rates (a) and cumulative mineralization (b) from soils amended with crop residues of wheat, pea and vetch, and non-amended soil (control) during 180-day field experiment under no-tilled conditions. Vertical bars in the graph indicate the minimum significant difference between crop species treatments (LSD at $P < 0.05$).

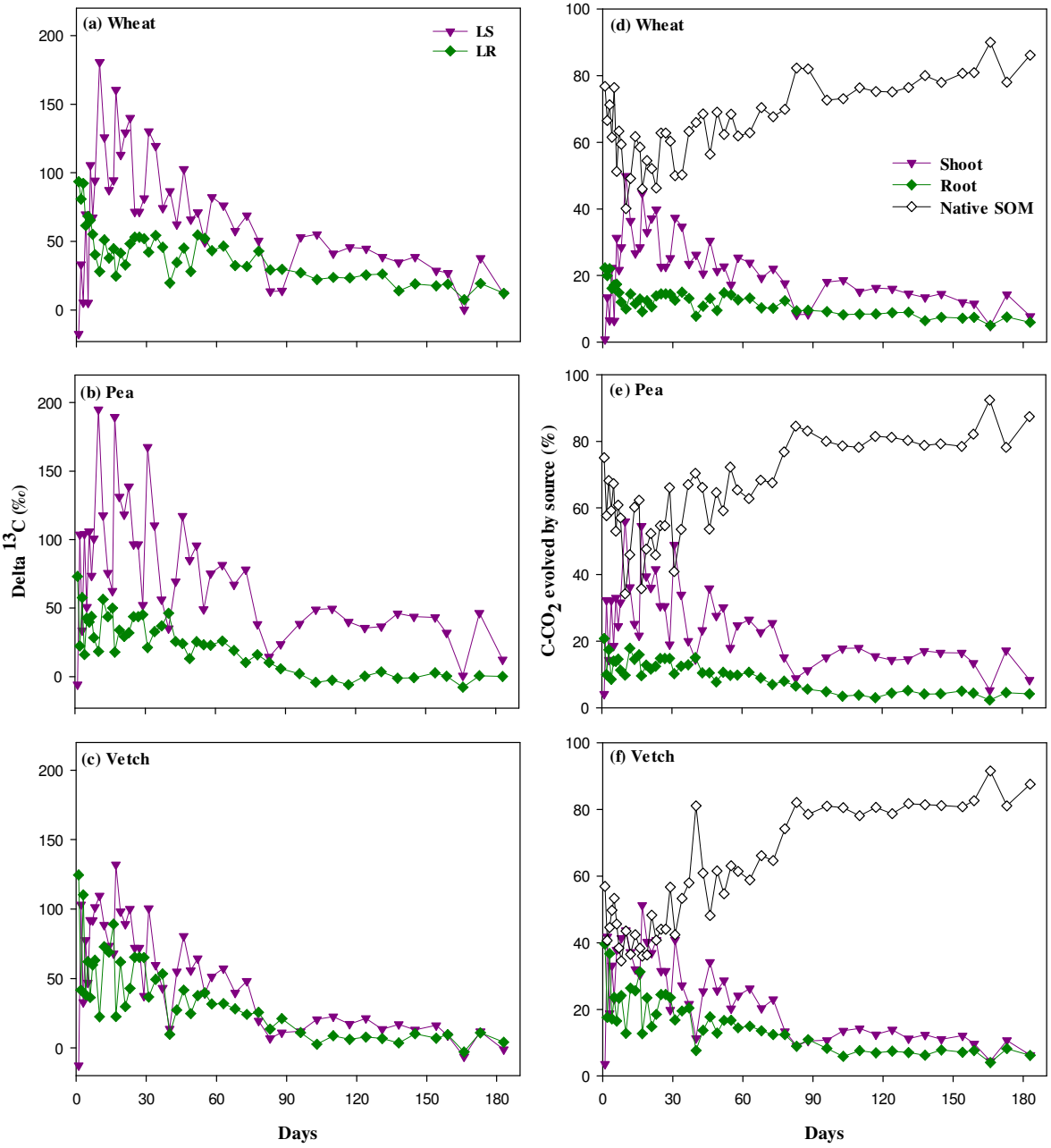

Fig. 4. $\delta^{13}C$ values (a, b, c) and relative contribution (% of total C-CO_2 evolved) from shoots, roots (roots+soil) of wheat, pea and vetch, and native soil organic matter (SOM) (d, e, f) during 180 days of decomposition under no tilled conditions. LS: labeled shoot, LR: labeled root (roots+soil). Values are the mean of 3 replicates.

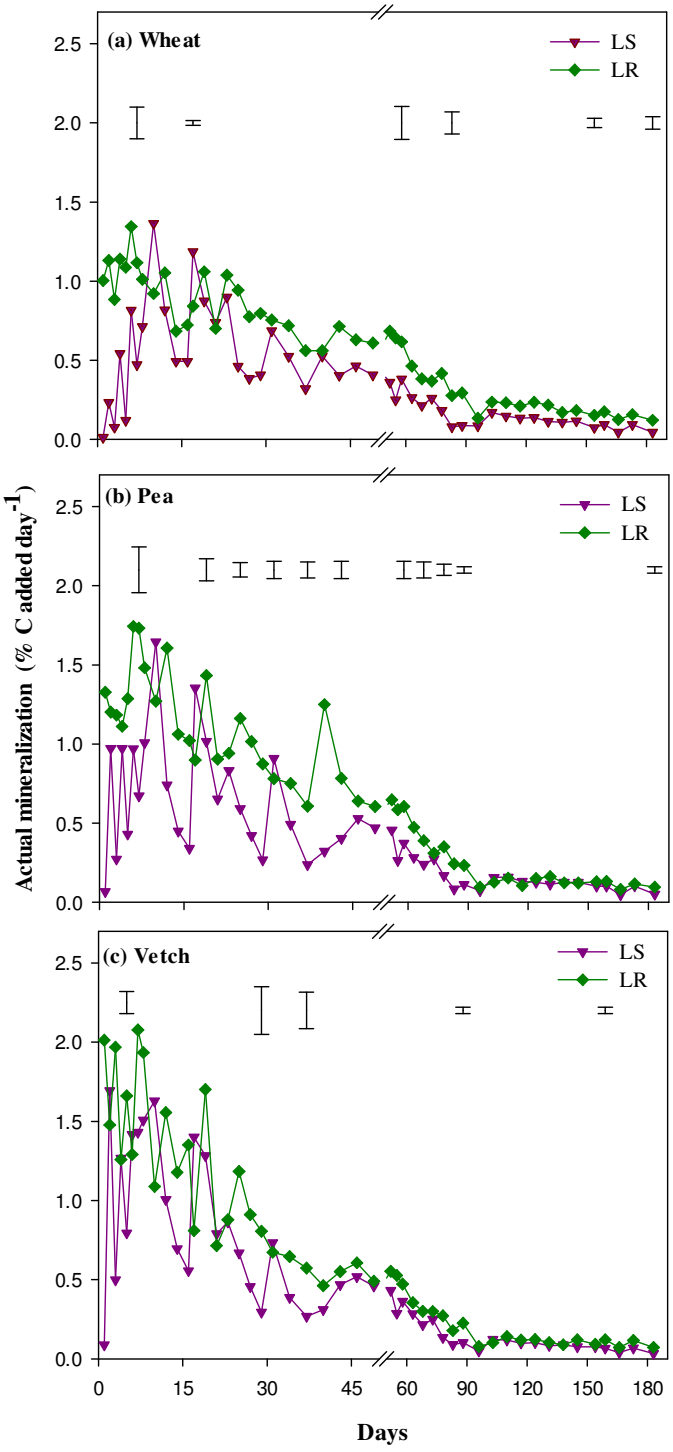

Fig. 5. Actual rates of C mineralization of shoots and roots of wheat (a), pea (b) and vetch (c) during 180 days of decomposition under field conditions. LS: labeled shoot, LR: labeled root (roots+soil). Values are the mean of 3 replicates. Vertical bars in the graph indicate the minimum significant difference between treatments (LSD at $P < 0.05$).

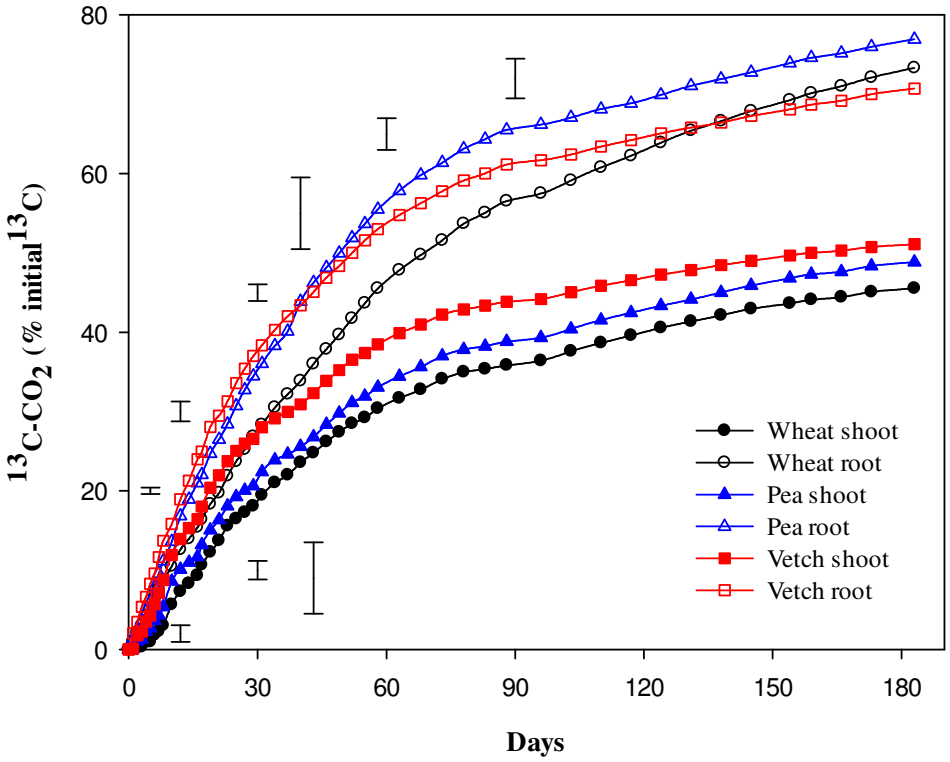

Fig. 6. Cumulative actual C mineralization of wheat, pea and vetch shoot and roots + soil residues calculated using ^{13}C measurements, during 180 days of decomposition under no tilled field conditions. Values are the mean of 3 replicates. Vertical bars in the graph indicate the minimum significant difference between treatments (LSD at $P < 0.05$).

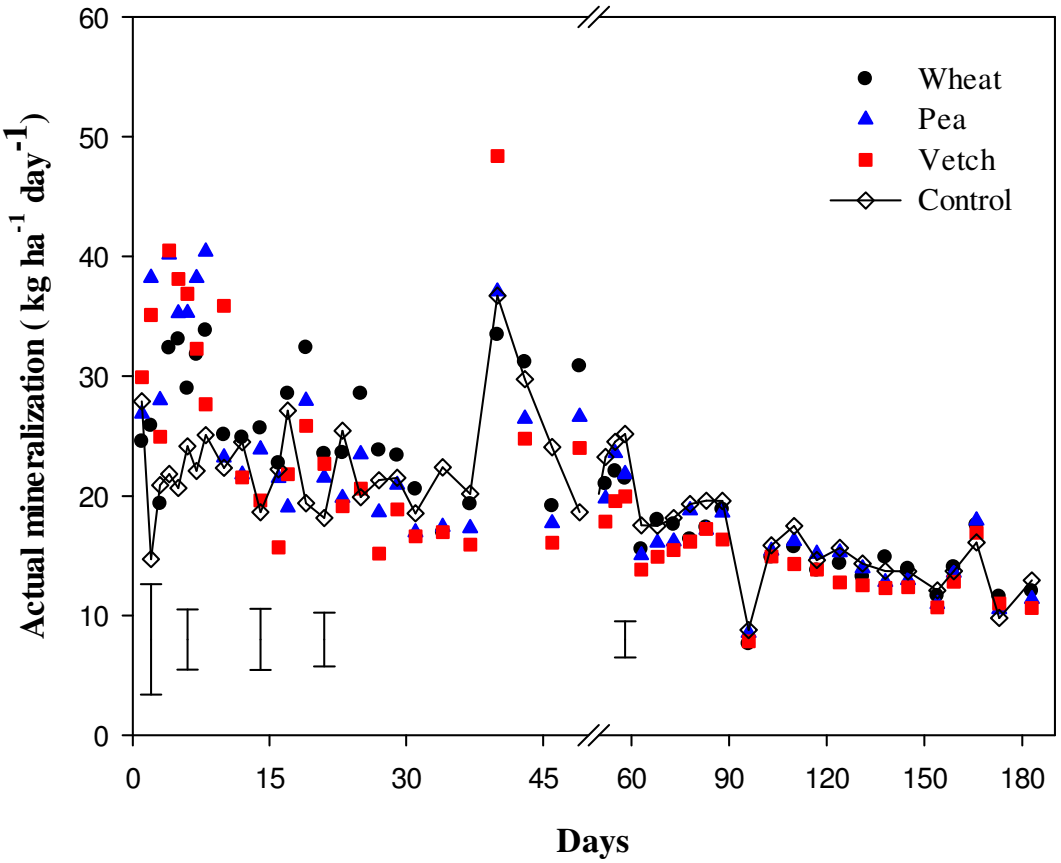

Fig. 7. Actual rates of C mineralization of native soil organic matter amended with wheat, pea and vetch residues during 180 days of decomposition under field conditions. The C-CO_2 derived from SOM in residues amended treatments was compared to C-CO_2 emitted from the non amended soil (control). Values are the mean of 3 replicates. Vertical bars in the graph indicate the minimum significant difference between treatments (LSD at $P < 0.05$).

Table 3

Distribution of ^{13}C in the various pools and ^{13}C balance at D_0 and D_{180} in soil with wheat, pea and vetch in labeled shoots and labeled root + soil treatments under no-till field conditions.

		Shoot (LS)		Roots + soil (LR)	
Crops	C pools	D_0	D_{180}	D_0	D_{180}
		% of initial ^{13}C			
Wheat	[1]residue^{13}C	100	36.2 ± 4.0	77.0	6.3 ± 3.5
	soil ^{13}C	0	2.3 ± 0.5	23.0	25.1± 2.2
	CO_2-^{13}C	0	45.5 ± 4.2	0	73.5 ± 10.3
	total	100	84.0 ± 4.2	100	104.9 ± 8.8
Pea	residue^{13}C	100	34.1 ± 2.4	72.8	2.8 ± 1.8
	soil ^{13}C	0	2.8 ± 0.7	27.2	21.5 ± 1.9
	CO_2-^{13}C	0	48.9 ± 2.9	0	76.8 ± 2.6
	total	100	85.8 ± 5.5	100	101.1 ± 5.6
Vetch	residue^{13}C	100	28.1 ± 1.7	76.4	2.4 ± 1.8
	soil ^{13}C	0	4.1 ± 0.9	23.6	29.5 ± 4.6
	CO_2-^{13}C	0	51.1 ± 1.7	0	70.8 ± 13.2
	total	100	83.3 ± 1.0	100	102.7 ± 17.1

[1]residue^{13}C: is the % of ^{13}C present in shoot or root residues.
D_0 = Day 0, D_{180} = day 180.

4 ARTIGO III – Carbon distribution in water-stable aggregates during the decomposition of ^{13}C-labeled shoots and roots of three winter crops under no-till field conditions[3]

4.1 Abstract

Objective The quality of plant residues predict their decay in soil. However, it remains unclear that how quality and location of the plant residues affect C retention in soil under no-till field conditions. The objective of this research were to track the relative contribution of root vs shoot derived C in soil aggregate as a function of their quality and location in soil.

Methods Wheat, pea and vetch plants were pulse labeled with $^{13}CO_2$ in the field and paired treatments were made by combining ^{13}C labeled shoots with unlabeled roots (LS) and unlabeled shoots with ^{13}C labeled roots (LR) (root+soil) to track shoot vs root residues C in whole soil and different aggregate size classes. Soil aggregates were separated by wet sieving.

Results A greater proportion of the shoot and root derived C_{new} of three crops was associated with large macroaggregates (LMa, >2000 µm) in 0-5 cm soil layer while small macroaggregates (SMa, 250–200 µm) held major proportion of root and shoot derived C_{new} in 5-15 and 15 -30 cm soil layers. The C_{new} in SMa and microaggregates (Mi, 53-250 µm) increased with time in all soil layers. The shoot derived C_{new} retained in soil was in order of vetch > pea > wheat while it was in order of vetch > wheat > pea for root residues. After 365 days, 30% of the root derived C was present in soil compared to 5 % (average of three crops) of the shoot derived C. The mean relative contribution of root vs shoot derived C_{new} was 2.1 ranging from 1.5 (pea) to 2.52 (wheat).

Conclusions Our results show that quality of residues impact C stabilization only when residues are decomposing in similar environmental conditions whereas, location of the residues prevail on quality under contrasted decomposition conditions and impact C stabilization in soil.

Keywords: ^{13}C distribution, No tillage, Roots, Shoots, Soil aggregates.

[3] Article is prepared in accordance with the format of the Journal *Plant and Soil*.

4.2 Introduction

Above- and belowground residues are the main source of soil organic matter (SOM) (Castellano et al. 2015) and their chemical composition directly affect their decay in soil (Aerts 1997; Silver and Miya 2001). However, the effects of residue quality on soil carbon (C) retention, storage and stabilization remain elusive and are inconsistent (Prescott 2010; Cotrufo et al. 2013). Some studies reported close relations between the initial chemical composition of residues and organic C retention in soil (Putasso et al. 2011), while others do not (Gentile et al. 2010, 2011). This suggest that quality of residues may not always be a major controller on the amount and form of residue derived C stabilized in soils by physical and chemical mechanisms (Kogel-Knabner and Kleber 2011; Schmidt et al. 2011). This scenario highlights the need to better quantify and understand the link between chemical composition of residues and C stabilization in soil.

Tillage management is the primary management practice that affects residue placement, decomposition and C stabilization in soil (Helgason et al. 2014). Under no-till systems, aboveground residues are at soil surface while roots are belowground, which may lead to different rates of decomposition and C stabilization in soil, independent of the quality of residues due to contrasted decomposition and stabilization conditions associated with the location of the residues. Earlier studies reported that root residues contribute more C to soil than shoot residues due to their recalcitrant nature i.e. slower decay in soil (Rasse et al. 2005). Recently, Cotrufo et al. (2013) proposed that high quality plant residues will contribute more to SOM due to high microbial substrate use resulting more microbial residues for physiochemical stabilization in soil. Few studies that have compared root and shoot residues decomposition under no-till field conditions reported faster decomposition of roots than shoot residues (Buyanovsky and Wagner 1987; Blenis et al. 1999), which means more microbial residues for stabilization than shoot residues at soil surface. This suggest that under contrasting conditions of decomposition, location of the residues prevail on quality of residues and ascribe C stabilization in soil. Therefore, there is a significant need for *in situ* studies to understand how the chemical quality and location of shoot and root residues decomposing at different locations affect C stabilization in soil.

The processes of plant residue C stabilization are closely related to soil structure. Soil aggregates are important agents of soil organic C (SOC) retention (Haile et al. 2008) and protection from microbial decomposition (Elliott 1986; Jastrow et al. 1996; Six et al. 2000). The dynamics of C derived from plant residues vary with aggregate size (Angers et al. 1997;

Majumder and Kuzyakov 2010; Schnitzer and Monreal 2011). Generally, organic C protected by the macroaggregates (Ma, >250 µm) shows a short-term storage, and the most stable C is stored in the smallest silt + clay size fraction (< 53 µm) (Six et al. 2002). Temporal changes in SOC are so slow to observe simply by measuring the total SOC in soil. Therefore, to link residue decomposition with C stabilization, it is required to know in which soil fraction residue C is stored (Hatton et al. 2014). Soil aggregate fractionation has been widely applied as a useful tool in studies related to organic matter stabilization by organo-mineral interaction and physical protection (Christensen 1992; Cambardella and Elliott 1994; Christensen 2001).

Detailed information about the *in situ* temporal variations during the simultaneous decomposition and stabilization of root and shoot residues C is challenging and is not possible with ordinary methods. *In situ* labeling of plants have been used to quantify the amount, and location of shoot and root residue-derived C in soil. Few field studies used this approach to track shoot and root residue derived C in soil while shoot and root residues were incorporated in soil (Puget and Drinkwater 2001; Williams et al. 2006; Kong and Six 2010). Although, these studies enhanced our understanding about relative contribution of root *vs* shoot residue C to SOM, definitive estimates of the relative contributions of root *vs* shoot residue C to SOM pools under actual no-till field conditions are yet to be determined.

In this study, we investigated how shoot and root residues of three different crops and their placement in soil affect residue derived C retention and stabilization under no-till field conditions. We pulse labeled wheat, pea and vetch plants with ^{13}C in the field during their vegetative growth, and then designed "paired" treatments by reciprocating labeled residues with unlabeled residues ($^{13}C/^{12}C$) to track root *vs* shoot derived C in different aggregate size classes, and (2) to evaluate the relative contribution of root *vs* shoot derived C to different aggregate size classes in function of their quality and placement in soil.

4.3 Materials and Methods

4.3.1 Experimental site

The field experiment was performed in the experimental area of the Departments of Soils, Federal University of Santa Maria (UFSM), located in Rio Grande de Sul (RS) State, Brazil (29°41′ S, 53°48′ W; approximately 90 m elevation). The soil is classified as Typic Paleudalf according to the USDA Taxonomy (Soil Survey Staff 2010) with 10% clay, 27% silt, 63% sand, 1.5 g.cm^{-3} bulk density and 6.6 g C kg^{-1} and -19.17 $\delta^{13}C$ in the top 0–30-cm soil layer.

The experimental area has a humid subtropical climate. The annual average temperature is 19.3°C, with the coldest monthly temperature below 9°C in June and the warmest above 30°C in January. The average annual precipitation is 1769 mm, without a dry season. The rainfall and air temperature during the present study period are shown in Fig 1. Prior to the experiment, the site had been under no-tillage winter [oat (*Avena sativa*) or vetch (*Vicia sativa*)]/summer [corn (*Zea mays*) or soybean (*Glycine max*)] crop rotation for 15 yr.

4.3.2 Experimental setup and ^{13}C labeling

On 15 May 2013, an area of 25 × 25 m^2 within the experimental site was marked, cleared and fenced to study the C dynamics of wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.) and vetch (*Vicia sativa* L.) root and shoot residues. The experiment was designed according to a split-plot design with crops as a main plots and with, or without ^{13}C pulse labeling as sub-plots. Within a given plot, two sub-plots were arranged in a randomized complete block design (RCBD) with three replications (3 crops × 2 sub-plots × 3 replications = 18 sub-plots). Inside each sub-plot, nine open-ended polyvinyl chloride (PVC) cylinders (35 cm height × 20 cm diameter, area of 0.0314 m^2) were hydraulically forced to a soil depth of 30 cm. The top of each soil cylinder was 5 cm above the soil surface with small holes on each side for water drainage. In addition, a total of twelve soil cylinders were similarly forced into soil in the area between each sub-plot as controls without plants. On 29 May 2013, eight seeds of wheat, pea and vetch were hand-sown in each soil cylinder of the sub-plots. After emergence, the plants in each cylinder were thinned to four plants for wheat, two plants for pea and four plants for vetch. Phosphorus and potassium were applied at a rate of 50 kg ha^{-1}. Nitrogen was applied at a rate of 90 kg N ha^{-1} for wheat in the form of ammonium sulfate in six equal doses (15 kg N ha^{-1}). All other standard and cropping practices were performed during the entire experiment.

The wheat, pea and vetch plants were pulse labeled with $^{13}CO_2$ between 22 June and 18 September, 2013. The ^{13}C-labeling pulses occurred weekly for 1.5 h between 9:00 and 11:00 in the morning. The labeling was continued up to the initiation of flowering, for a total of eleven labeling pulses. To label the plants, the labeled sub-plots were enclosed in labeling chambers. $^{13}CO_2$ gas was generated through the reaction between 1 mol L^{-1} solution of $NaH^{13}CO_3$ (33 atom %) (Cambridge isotope laboratories, Inc, USA) with 2 mol L^{-1} HCl in a beaker placed inside the labeling chamber. The total CO_2 concentration in the labeling chamber was monitored using a portable infrared gas analyzer (IRGA, model SD800, Extech, USA). The CO_2 concentration in the chambers was maintained at approximately 266 to 400

ppm, and the $NaH^{13}CO_3$ (33 atom %) solution was added when the CO_2 levels dropped to 266 ppm during 1.5 h of labeling. Ice packs were used to minimize excessive heating and to condense excess humidity during the ^{13}C labeling. After each labeling pulse, the chambers were replaced over the labeled sub-plots at sunset to capture overnight $^{13}CO_2$ respiration. The chambers were removed the following morning after the CO_2 levels fell below 266 ppm. The plants were harvested on 2 October 2013 and dried to a constant weight at 40°C for 48 h in a forced-air oven.

4.3.3 ^{13}C paired treatments

We designed "paired" treatments with ^{13}C labeled and unlabeled wheat (W), pea (P) and vetch (V) shoots and roots, similar in chemical composition except for the ^{13}C enrichment (Table 1). The design consisted of two treatment combinations for each crop: ^{13}C-labeled shoots + unlabeled roots (LS) and unlabeled shoots + ^{13}C-labeled roots+soil (LR). These treatment combinations resulted in six treatments: WLS and WLR for wheat, PLS and PLR for pea and VLS and VLR for vetch. In the WLR, PLR and VLR treatments, the soil in the cylinders was enriched with ^{13}C prior to the start of the decomposition experiment due to the plant exposure to $^{13}CO_2$ during vegetative growth. The root+soil inside the labeled and unlabeled cylinders remained undisturbed. Destructive sampling of three labeled and unlabeled cylinders per crop was performed at the start of the decomposition experiment (day 0 = D_0) to determine the initial ^{13}C (C_0) in the soil and roots, and these values were used for subsequent calculations. The initial soil ^{13}C in the labeled cylinders corresponded to C from rhizodeposition during plant growth. Three bare soil cylinders not cropped or labeled with ^{13}C were also sampled at D_0 and used as the control for ^{13}C calculations.

Dried stems were cut into 10 cm pieces, and dried leaves were used intact. The "shoots" were prepared as a mixture of the stems and leaves of each species, according to their actual mean proportion, which was determined for the plants at harvest time. The shoots were placed on the soil surface inside the soil cylinders at a rate of 5 Mg ha^{-1}. The labeled shoot treatments (WLS, PLS and VLS) were prepared by placing the labeled shoots collected from the labeled cylinders of each species on the surface of the prior unlabeled soil cylinders, i.e., containing unlabeled roots+soil. Similarly, the labeled roots+soil treatments (WLR, PLR and VLR) were prepared by placing the unlabeled shoots collected from the unlabeled cylinders on the soil surface of the prior labeled cylinders, i.e., containing labeled roots+soil. For all treatments, the soil and roots remained undisturbed until the cylinders were sampled.

4.3.4 Cylinders sampling

Three randomly selected soil cylinders from the labeled and unlabeled sub-plots were destructively sampled on days 0, 60, 180 and 365 after collecting surface residues by excavating the cylinders to a depth of 35 cm. The soil cylinders were placed in plastic bags, transferred to the laboratory, stored at 4°C and processed within 2 days. The entire soil core (0-30 cm) was removed by cutting the soil cylinder at both sides using an electric saw. The soil core was then separated into three soil layers (0–5, 5–15 and 15–30 cm), which were weighted. All visible roots were immediately removed by hand from each soil layer. The moist soil layers with preserved structure were manually broken along their plane of weakness to obtain soil aggregates that pass through an 8-mm sieve. The aggregates were air-dried and stored in crush resistant airtight containers at room temperature.

4.3.5 Aggregate size fractionation

Aggregates were separated by wet sieving following the method described by Angers et al. (2008) by using a modified Yoder (Yoder 1936) wet-sieving apparatus as described by Mikha et al. (2005). Four aggregate size classes were separated: i) large macroaggregates (LMa, >2000-µm), ii) small macroaggregates (SMa, 250-2000-µm), iii) microaggregates (Mi, (53-250-µm) and iv) silt and clay (<53-µm). A set of two sieves (2000>250) was placed inside the oscillation cylinders. The sieves were set at the highest point and the oscillation cylinders were filled with water to the point, where the bottom sieve (250 µm) was completely covered with water, without reaching the top sieve (2000 µm). Air dried aggregates (<8.00mm), about 40 g in duplicates were evenly distributed on top of the 2000 µm sieve and water was added until the mesh of the top sieve (2000 µm) was in level with water surface. The aggregates were then allowed to wet by capillarity at room temperature for 10 min before the start of the wet-sieving. The apparatus specifications of oscillation time (10 min), stroke length (3.7 cm), and frequency 30-cycle min^{-1} were held constant. After wet sieving, soil plus water remaining in the oscillation cylinder was poured onto the finer sieve (53 µm) and the water was collected in plastic buckets and left undisturbed for 72 hrs. The silt + clay (<53 µm) were collected by removing supernatant by siphoning. Material remaining on each sieve was backwashed into pre-weighed aluminum pans and dried at 50 ^{o}C (24 h) and weighed. Subsamples of aggregates from each size class were dried at 105 ^{o}C for 24 h to allow correction for dry weight.

Subsamples from each size fraction were ground in a steel ball mill for the determination of total C and $\delta^{13}C$.

4.3.6 Analysis of C content and $\delta^{13}C$ values

Total carbon content and ^{13}C content of bulk soil and aggregate size fractions were measured by using an isotope ratio mass spectrometer (Delta V Advantage, IRMS Thermo Fisher Scientific Inc. Germany) coupled with an elemental analyzer (Flash 2000, ThermoEletron Corporation, Breman, Germany) by an interface (ConFlow).The isotopic values are expressed relative to the Vienna-Pee Dee belemnite (V-PDB) reference as $\delta^{13}C$:

$$\delta^{13}C(‰) = \left(\frac{R_{sample}}{R_{V-PDB}} - 1\right) \times 1000 \quad (1)$$

$$^{13}C(\text{atom }\%) = 100 \times \frac{\left((\delta^{13}C+1000)\times R_{V-PDB}\right)}{\left((\delta^{13}C+1000)\times R_{V-PDB}+1000\right)} \quad (2)$$

$$^{13}C \text{ excess (atom \%)} = {}^{13}C \text{ sample(atom \%)} - {}^{13}C \text{ natural abundance (atom \%)} \quad (3)$$

where R_{sample} is the ratio of $^{13}C/^{12}C$ in sample and $R_{V\text{-}PDB}$ is equal to 0.011179. ^{13}C (at %) represents the percent of ^{13}C atom in total carbon atoms and ^{13}C excess (at %) is the difference in the ^{13}C content between a labeled sample and a non-labeled sample (i.e., a sample at natural abundance).

4.3.7 Calculations and statistics

LR (roots+soil) treatments had ^{13}C in soil prior to the start of the experiment (day 0) due to ^{13}C pulse labeling, which was not the case for the LS treatments. Therefore, we measured the amount of ^{13}C in roots ($C_{0\ root}$), shoots (C_{0shoot}) and soil (C_{0soil}) in both labeled and unlabeled sub-plots to calculate the total ^{13}C ($C_{0\ total}$).

$$C_{0\ total} = C_{0\ root} + C_{0\ shoot} + C_{0\ soil} \quad (4)$$

C_{0total} was 395, 264 and 418 mg $^{13}C\ m^{-2}$ in the LR (roots+soil) treatments and 997, 959 and 727 mg $^{13}C\ m^{-2}$ in LS treatments for wheat, pea and vetch, respectively. Because the C_0 total

values were different in the two treatments, comparisons between the treatments were made on a percentage basis.

Total C in whole soil and each aggregate size class was calculated by multiplying the proportion of that aggregate size in bulk soil by the carbon concentration of the associated aggregate size class. The following equation was used to estimate the amount of ^{13}C incorporated into whole soil and various aggregate size classes:

$$^{13}\text{C amount}(\text{mg m}^{-2}) = {}^{13}\text{C excess (atom \%)} \times \text{C pool size (mg m}^{-2})/100 \quad (5)$$

where C pool size is the carbon content in whole soil and aggregate size classes.

The recovery (%) of shoot or root -derived ^{13}C in whole soil and all aggregates after wet-sieving was calculated by dividing the amount of ^{13}C in whole soil and all aggregate size classes by the amount applied at day 0 ($C_{0\ total}$). C_{new} in whole soil and aggregates size classes was calculated by following equation:

$$C_{new}(\text{mg m}^{-2}) = {}^{13}\text{C recovered (\%)} \times \text{total C applied (mg m}^{-2})/100 \quad (6)$$

The relative contribution factor (RCf) of root *vs* residue C was calculated by following equation as suggested by Rasse et al. (2005):

RCf = [(root-derived soil C/total root C input)/(shoot-derived soil C/total shoot C input)] (7)

All results were shown as means of three replicates with standard deviation. Whole soil C_{new}, C_{new} in aggregate size classes for each crop at one sampling time, between an aggregate size class for all sampling times in three soil layers were analyzed by using ANOVA. Differences between means were calculated based on least significant difference (LSD) test (Table 2). The level of significance of statistical test was $P < 0.05$ in this study.

4.4 Results

4.4.1 Climatic conditions during the experiment

Southern Brazil has a humid subtropical climate associated with high summer temperatures and heavy rainfall; over 60% of the annual rainfall occurs during summer months. Although the monthly distribution of rainfall is quite uniform, day-to-day variability is high and occasionally rainfall exceeds 100 mm day^{-1}. The cumulative rainfall during the decomposition experiment was 2189 mm, and the average daily air temperature varied between 23°C and 17°C during 1 yr (Fig. 1).

4.4.2 Aggregate size distribution

Aggregate size distribution and total C content (%) did not differ significantly between the "paired" treatments for wheat (WLS vs. WLR), pea (PLS vs. PLR) and vetch (VLS vs. VLR) at any time during 365 days of decomposition (data not shown). Therefore, the aggregate size distribution and total C content in "paired" treatments for each crop were presented as an average of the "paired" treatments (Table 3, Appendix 1). The recovery of the soil after wet sieving ranged from 97 to 99 % of the whole soil. In the 0-5 cm soil layer, water-stable LMa, constituted the highest quantities (52–80%) soil weight) followed by SMa (12–25%) and Mi (7–19%) for the three crops during 365 days. The LMa were increased by 16 and 19 % in pea and vetch during the first 60 days and then declined during the reaming of the experiment. An opposite trend was observed for SMa and Mi. After a decrease in the first 60 days, SMa were increased by 6, 8 and 14 % whereas Mi were increased by 9, 8, and 8 % for wheat, pea and vetch, respectively. In 5-15 and 15-30 cm soil layers, SMa, constituted the highest quantities (50–64%) followed by Mi (19–37%). In the 5-15cm soil layer, LMa were decreased by more than 50% of the day 60 while Mi were increased by 2, 4 and 9 % for wheat, pea and vetch respectively, with little or no change in SMa and silt + clay size classes after 1 yr. In the 15-30 cm soil layer, the only significant differences were found in LMa and Mi in the last sampling. The lowest proportion (2–5%) of soil was found in silt + clay fraction in all soil layers and largely remained unaffected.

4.4.3 C_{new} in whole soil, relative contribution and recovery

Shoot derived C_{new} in the whole-soil (0-30 cm) among the three crops was significantly different after 365 days (Fig. 2a) and was in order of vetch > pea > wheat while root (root+soil) derived C_{new} in the whole-soil was not significantly different between crops for any sampling time and it was in order of vetch > wheat > pea (Fig. 2b). The relative contribution of root (root+soil) *vs* shoot C_{new} in whole soil was neither significant for a given crop between sampling times nor between crops for the same sampling time (Fig. 2c). The relative contribution of root (root+soil) *vs* shoot derived C_{new} decreased with time and this decline was more prominent in pea and vetch than wheat. The relative contribution of root (root+soil) *vs* shoot derived C_{new} was highest in wheat (2.52), intermediate in vetch (2.25), and lowest in the pea (1.50). After 365 days, approximately 30 % of wheat, pea and vetch root ^{13}C was recovered (Fig. 3b) in whole soil (0-30 cm) compared to 4 % of wheat, 5 % of pea and 6 % of the vetch shoot applied ^{13}C (Fig. 3a).

4.4.4 C_{new} in aggregates

Root labeled treatments had C_{new} in soil prior to the start of the experiment in the form of rhizodeposits due to the pulse labeling of plants compared to shoot labeled treatments (Table 1). Since it was difficult to partition C_{new} derived from rhizodeposits and labeled roots during the experiment. Therefore to overcome this difficulty, we estimated C_{new} from rhizodeposits in labeled root treatments at D_0 and included them with total C applied with roots. Consequently, C_{new} derived from roots includes both the decomposition of rhizodeposits and roots (root+soil) afterwards, while C_{new} derived from shoot residues was comprised of shoots decomposition. On D_0, the highest C_{new} from rhizodeposits was measured in LMa in all treatments in 0-5 cm soil layer, and ranked in the order of LMa > SMa > Mi > silt +clay fraction (Table 5). In the 5-15 and 15 to 30 cm soil layers the C_{new} from rhizodeposits was in the order of SMa > LMa > Mi > silt +clay fraction (Table 5).

4.4.5 Shoot and root derived C_{new}

Shoot derived C_{new} in different aggregate size classes over time is presented in table 4. Among aggregate size classes, most of the shoot derived C_{new} retained in LMa in 0-5 cm, and in SMa in lower (5-15 and 15-30 cm) layers followed by Mi, and was in order of vetch > pea > wheat.

In the 0-5 cm soil layer, C_{new} in LMa was maximum at 60 days and thereafter declined with a concomitant increase of C_{new} in SMa, Mi and silt + clay fraction until the end of experiment. However, the increase was only significant for Mi. In the lower soil layers (5-15 and 15-30 cm), C_{new} in all aggregates size classes declined between 60 and 180 days followed by an increase in SMa, Mi and silt + clay. Lowest shoot derived C_{new} was recovered in silt + clay fraction of the three soil layers.

The contribution of root (root+soil) derived C_{new} after 365 days varied with aggregate size, soil layers and crop types (Table 5) and was in order of vetch > wheat > pea. Similar to shoot derived C_{new}, majority of the root derived C_{new} (root+soil) was also retained in macroaggregates (Ma) (LMa + SMa) in all the three soil layers, followed by Mi. Over the time, C_{new} was increased in SMa and Mi with a concomitant decrease in LMa between day 60 and 365 for all crops in all soil layers except for pea in Mi in 5-15 and 15-30 soil layers. Among the three soil layers, C_{new} as sum of all aggregates size fractions was in the order of 0-5 > 5-15 > 15-30 cm.

4.5 Discussion

We addressed how differences in quality and placement of shoot and root residues under no-tillage (no-till) system affect C distribution in soil. Since the field study involved *in situ* roots and "paired" treatments. The observed pattern of ^{13}C recovery in whole soil and soil aggregate size classes in LR treatments was ascribed to the combined effect of root + rhizodeposits decomposition during the experiment. In contrast, this was not the case with shoot residues, therefore the recovery of ^{13}C in LS treatments was only due to the decomposition of the shoot residues. We used wet sieving fractionation technique to quantify how the labeled C from shoot and root residues was allocated to different aggregate size classes.

4.5.1 Aggregate size distribution

Our results and the previous findings (Harris et al. 1966; Gale et al. 2000) suggest that addition of plant residues increased macroaggregation up to a point followed by a decline thereafter. The temporary increase is apparently due to the labile C that provides the basis for the initial formation of Ma. However, as the labile SOC pool is utilized and microbial activity decreases, Ma loses stability, eventually disrupting and releasing more stable Mi (Jastrow et al. 1998; Six et al. 1999). The observed differences between the crop species suggest that

quality of residues affect the dynamics of water-stable macroaggregation (Martins et al. 2013), since high quality residues are postulated to increase the rate of Ma turnover (Six et al. 2001). However, this seemed to be only short-term as the differences among the plant residues became smaller with time. In our experiment, roots were intact, therefore we cannot overlook the effect of rhizodeposition, fine root turnover and decomposition on aggregate formation. Additionally, the formation and stabilization of Ma have been found to be positively correlated with soil organic C (SOC) content (John et al. 2005) and soil microorganisms, especially biomass of fungi (Helfrich et al. 2008, Abiven et al. 2007) which has been reported to be abundant when residues were placed on the soil surface compared to incorporated residues (Holland and Coleman 1987). The here observed sharp increase in LMa in 0-5 cm soil layer compared to blunt increase in 5-15 cm layer and with no significant change in 15-30 cm soil layers during the same time support this hypothesis. Gale et al. (2000) also reported that surface residues play a minor role in Ma formation deeper in the soil profile under no-till management.

4.5.2 Shoot and root derived C_{new} in aggregates

The distribution of shoot and root derived C followed a similar pattern during simultaneous decomposition at different positions under no-till field conditions and was in the order of Ma > Mi > silt +clay fraction in all three soil layers. LMa were the most prominent C storage location for both shoot and root residues in 0-5 cm soil layer, while SMa in lower layers (5-15 and 15-30 cm). On an average of the crops and soil layers, Ma (LMa + SMa) retained up to 64 % and 80 % of the C_{new} derived from shoot and root residues, respectively after 1 yr. This finding highlights the importance of Ma as the most important C storage location of shoot and root derived C under no-till field conditions in subtropical sandy loam soil. The higher retention of C_{new} in the Ma was possibly be due to the formation of more particulate organic C in the Ma than in the Mi. Our results agree with the findings of Olchin et al. (2008) from a 1 yr long *in situ* decomposition experiment with ^{13}C labeled wheat shoot residues under no-till conditions. They found for water-stable aggregates, a higher percentage of wheat shoot-derived C in Ma. With the time, C_{new} in LMa decreased whereas C_{new} in SMa and Mi increased significantly, with few exception for root residues in 5-15 and 15-30 cm layers. The variability in C_{new} of the two soil layers (5-15 and 15-30 cm) could also be due to the leaching of dissolved organic C from upper soil layer which complicated our results. It seems likely, that a significant amount of C could be transported deeper in the soil through leaching in our

experimental conditions, especially after every rainfall events. However, in general our results agree with those of Angers et al. (1997) who observed that, when labeled wheat straw was incorporated into the soil, the concentration of newly added C in Ma was initially high and then declined, but the concentration of C_{new} in Mi increased with time. Similarly, Gale et al. (2000) also observed that the concentration of ^{14}C in Mi increased with time with a corresponding decrease in Ma under simulated no-till laboratory experiment.

Since the SOC contained in macroaggregates was younger and more labile than SOC in Mi (John et al. 2005) and its decomposition resulted in the disintegration of LMa with time due to the mineralization of the organic binding agents. Therefore, the increasing amount of C_{new} in Mi with time appeared to be the under combined influence of the C_{new} entering from the decomposition of C in LMa and increasing amount of soil mass in this fraction. It has been reported that the amount of C_{new} in smaller aggregates fractions increases as long as the LMa can provide C_{new} (Aita et al. 1997). Although the C_{new} in LMa decreased with time but it remained higher than smaller fractions even after 365 days. We believe that the higher C_{new} in Ma after 1 yr of field decomposition was due to the continuous incorporation of newly derived shoot and root C. In all soil layers, lowest C_{new} was found in the silt + clay fraction. This fraction contained no sand particles but it might be that the low C_{new} in this fraction was influenced by low clay contents and the accumulation of free silt particles with low or no binding capacity of SOC (e.g. small quartz grains) (John et al. 2005). Several tracer studies have indicated that the composition and turnover of the <50-µm fraction is very heterogeneous (Aita et al. 1997).

4.5.3 Root *vs* shoot C_{new}

The amount of C derived from shoot and root residues was different. Earlier studies suggest that root C stabilized preferentially in soil compared to shoot C (Gale et al. 2000; Puget and Drinkwater 2001; Rasse et al. 2005; Kong and Six 2010) with which our findings agreed. Although more than 2 (vetch), 3 (wheat) and 4 (pea) times more shoot C than root C (root+soil) was added, only 8.9 (wheat), 11.6 (pea) and 14.3 (vetch) g C_{new} m^{-2} of shoot C *vs* 23.8 (wheat), 18.3 (pea) and 32.0 (vetch) g C_{new} m^{-2} of root C (root+soil) remained in the whole soil after 1 yr. Kong and Six (2010) reported 52% recovery of root-derived C *vs* only 4% recovery of hairy vetch shoot-derived C in the soil after one growing season. Similarly, Puget and Drinkwater (2001) also observed 50% recovery of root-derived C *vs* only 13% recovery of hairy vetch shoot-derived C in the soil after one growing season. In Puget and

Drinkwater (2001) and Kong and Six (2010) experiments, shoot and roots were incorporated in soil which was not the case in our experiment. Therefore, we believe that, differences in the decomposing conditions and stabilization mechanisms may explain the differences in amounts of shoot *vs* root derived C_{new} in soil.

The initial location of the crop residues differ in the way the C enters in the soil. The shoot residues were left on the soil surface, decomposed slowly than root residues (Artigo 2). This was also observed by the greater amount of C remaining in shoot residues than root residues (c.a.17 *vs* 1 %) after 1 yr. Gale et al. (2000) proposed that C from surface residues may enter the soil in two different ways: as fungal biomass into the soil via hyphal bridges or as small bits of residue that fell to the soil surface as decomposition proceeded. Additionally, when the residues were left on the soil surface, some of the residue C enters in the soil by diffusion at the soil-residue interface near the soil surface (Gaillard et al. 1999) and by convective transport after rain (Coppens et al. 2006). Consequently, C originating from surface shoot residues would lack the physical protection that root C has (Menichetti et al. 2015).

On the other hand, greater stabilization of root C was attributed to the: (i) the slower decay of roots than shoots, (ii) physical protection within soil aggregates, and (iii) the continuous nature of root C inputs (Rasse et al. 2005). Our results are contradictory to the first rationale of Rasse et al. (2005). In our study, roots decomposed faster than shoot residues and yet contributed more C_{new} to SOM. Generally, slower decay of roots than shoots is believed to be due to greater chemical recalcitrance of the root residues. However, slower decay of root *vs* shoot implies that, all conditions being equal, root residues decomposed at a slower rate in soil than their shoots (Rasse et al. 2005). Measuring this effect implies that root and shoot residues are subjected to equal conditions in soils, which was not the case in our experiment i.e. shoots were on the soil surface and roots were in the soil. This shows that location of the residues primarily affect decomposition rather than their chemical quality when they were not subjected to similar conditions. Our results show that root residues can decompose faster in the soil than their shoot counterparts at the soil surface and greater stabilization of root C is due to their close proximity to the soil matrix instead of their greater chemical recalcitrance. Close interaction of root residues with the soil minerals has been suggested to be the main soil-specific protection pathway for root C (Balesdent and Balabane 1996; Oades 1995). Our data showing greater root C (root+soil) *vs* shoot C recovery in all soil aggregate size classes supported the second rationale in Rasse et al. (2005) for the greater retention of root-derived C than residue-derived C. The greater association of root-derived C with the Ma (LMa + SMa)

in our study supports the notion that the existence of stable Ma in soil is very important for the stabilization of SOM, because the formation of stable Mi is fostered within Ma (Rasse et al. 2005). Since we measured the rhizodeposits in root labeled treatments after harvest, our data showing C_{new} at day 0 in all aggregate size classes in all soil layers confirms the third rationale in Rasse et al. (2005). Another explanation of the higher root derived C_{new} in soil is the stabilization and protection of the labile root C (rhizodeposits) during the growth and early stages of decomposition. Schmidt et al. (2011) proposed that SOM persistence in soil is due to environmental factors that reduce decomposition than to intrinsic properties of the organic matter itself.

The RCf of vetch root (root+soil) *vs* shoot derived C_{new} for hairy vetch in our experiment was low (2.5 *vs* 3.7) compared to that obtained by Puget and Drinkwater (2001). The Puget and Drinkwater (2001) did not considered C input from rhizodeposition which may led to a higher relative contribution estimate. Similarly, higher recovery of root derived C_{new} (50-52 %) by Puget and Drinkwater (2001) and Kong and Six (2010) in comparison with our experiment (c.a.30 %) could be due to the exclusion of C input from rhizodeposition to the total C added from roots. The mean relative contribution factor for root *vs* shoot derived C calculated by Rasse et al. (2005) was 2.4 ranging from 3.7 to 1.5 for *in situ* experiments in which our values also falls.

4.5.4 Residue quality and C_{new}

The formation of SOM is related to the microbial decomposition of plant residues which depends on residues chemical quality and the subsequent physio-chemical stabilization of the microbial residues. High quality residues decomposes fast (i.e., labile) and 'low-quality' residues decomposes slowly (i.e., recalcitrant). Earlier it was believed that low quality residues decompose slowly and contribute more to SOM formation (Rasse et al. 2006). However, recently Cotrufo et al. (2013) proposed that high quality residues produce more microbial residues than low quality residues which provides more SOM stabilization opportunities. Therefore, more SOM would be formed from high-quality residues compared to low-quality residues. Our findings in the present experiment support this hypothesis when we compared roots and shoots separately among crops. Though, three different type of crop plants were used in this study, unfortunately little differences were observed in the chemical composition of the plant residues after harvesting. Besides the less variability in chemical composition, we observed that vetch shoot and root residues which were the high quality

residues in our experiment contributed more C_{new} compared to pea and wheat after 1 yr, though not different significantly. Another important finding in our experiment which does not corroborate with the speculation of Cotrufo et al. (2013) is the less contribution of pea roots C_{new} to SOM compared to wheat roots which are generally known as low quality residues than pea roots. Although, pea roots had low C:N ratio, high soluble C compared to wheat roots, we believe that the low quantity (865 kg ha^{-1}) of pea root residues lead to the reduced contribution of C_{new} to SOM compared to wheat roots (1319 kg ha^{-1}). It was earlier reported that litter quantity rather than quality is the main determinant of the amount of physico-chemically stabilized SOM (i.e. Castellano et al. 2015). The C_{new} derived from the shoot residues of the three crops was in the order of vetch > pea > wheat which confirms the proposition of Castellano et al. (2015) that when equal rates of high- and low-quality residues are added, C_{new} was always greater in soil having high quality residues thereby confirming the hypothesis of Cotrufo et al. (2013).

On the other hand, when we compared root *vs* shoot C addition to SOM, our results does not corroborate with the hypothesis of Cotrufo et al. (2013) in terms of quality of residues, since roots are known as low quality residues than shoot residues. However, our results support the speculation of Cotrufo et al. (2013) in terms of microbial substrate use. Castellano et al. (2015) proposed that plant residues that produce more microbial residues result in more organic matter that can be physico-chemically stabilized. In our experiment, roots decomposed faster than shoot residues which means that root residues produced more microbial residues than shoot residues and hence more C to SOM which confirms the notion of Castellano et al. (2015). This was observed by greater relative contribution of root *vs* shoot derived C in our experiment. Putasso et al. (2011) concluded that initial chemical properties of plant residues have a greater impact on C stabilization in tropical sandy soils. Our results show that quality of residues impact C stabilization only when residues are decomposing in similar environmental conditions i.e. high quality residues contribute more C in soil. In contrast, position of the residues prevail on quality, through its influence on decomposition and impact C stabilization in soil i.e. greater decomposition and stabilization of root C being in the soil compared to less decomposition and stabilization of shoot C being at soil surface.

4.6 Conclusions

The use of labeled shoot and root residues showed that the differences in the position lead to the differences in the fate and stabilization root *vs* shoot C in whole soil and soil aggregates

after 1 yr under no-till field conditions. A greater amount of root and shoot derived C_{new} was associated with Ma which declined gradually, whilst it steadily increased in Mi. On the other hand, slight temporal variation was observed in silt + clay fraction. By the end of 1 yr, on an average of three crops 30 % of the root derived C_{new} was present in whole soil compared to 5 % of shoot derived C_{new}. This illustrates the importance of greater physical contact with residues, since roots were in soil. We conclude that the residue (root and shoot) C_{new} was stabilized gradually in soil by the incorporation of C from decomposing residues with time in soil aggregates and by the redistribution of C_{new} from Ma to smaller aggregates. The isotopic labeling of crop plants is a useful tool to study the C dynamics of shoot and root derived C under no-till field conditions and has important implications for developing management strategies e.g. selection of crops to include in crop rotations to optimize C sequestration in soil.

Acknowledgements

The first author's doctoral study was funded by a CNPq-TWAS postgraduate fellowship program. This work was supported by the Brazilian government through the Conselho Nacional de Desenvolvimento Científico e Tecnologico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS). We thank André Friderichs and Fabiano Halmenschlager for the ^{13}C isotopic analysis.

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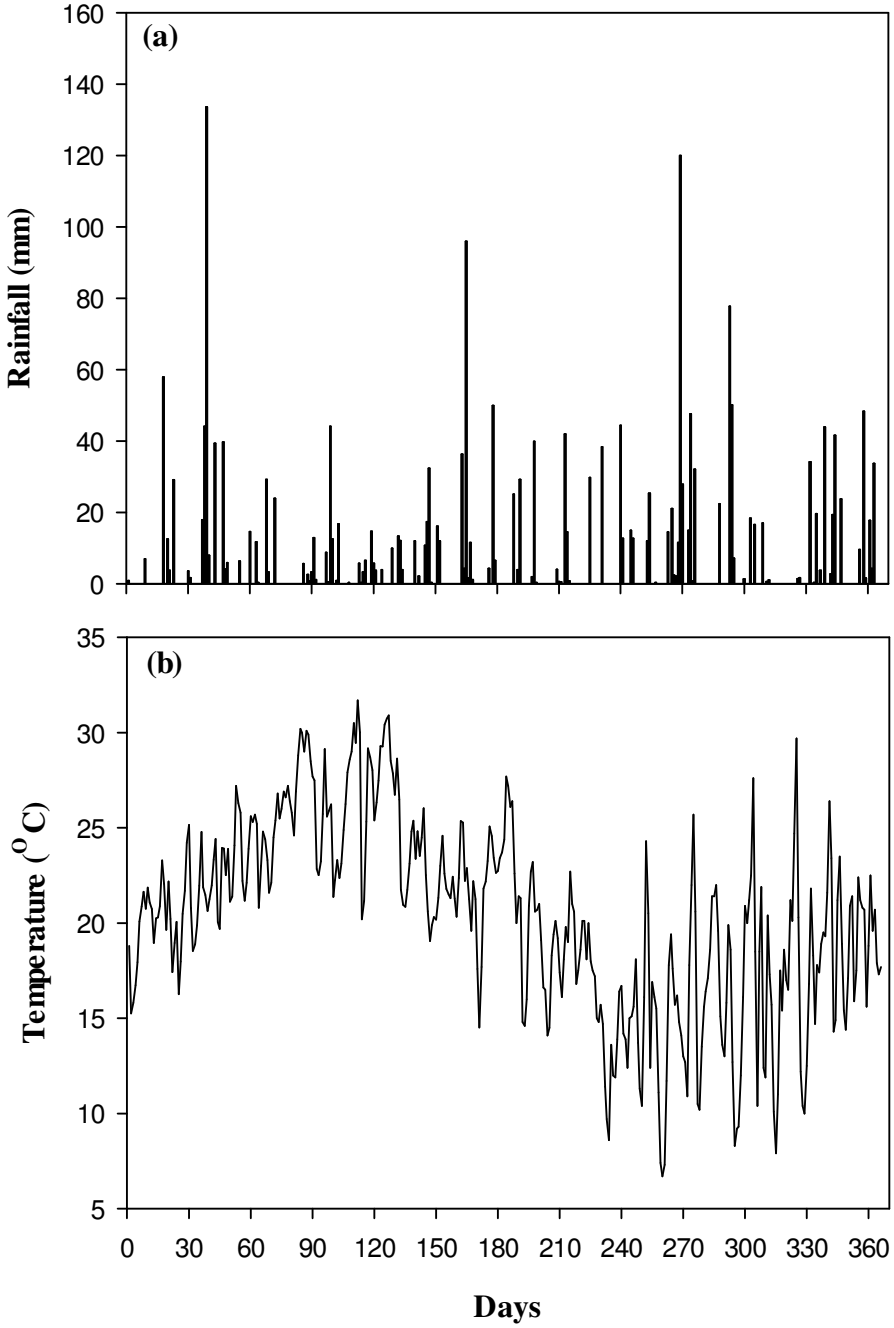

Fig. 1 Daily rainfall (a) and mean air temperature (b) during the decomposition of shoot and root residues under no-till field conditions at Santa Maria, Rio Grande do Sul, Brazil. Day 0 is the start of the field decomposition study (04-10-2013)

Table 1 Amounts of Dry matter (DM), Carbon (C) and Nitrogen (N) added, initial chemical composition of the shoots and roots and $\delta^{13}C$ of shoots, roots residues and soil after pulse labeling with ^{13}C under no-till field conditions

			Residues										**Soil (0 -30 cm)**		
Crops	**Treatments**	**Combination**	DM	C	N	C/N	$\delta^{13}C$	SOL	CEL	HEM	LIG	$^{13}C_{residues}$ [2]	$\delta^{13}C$-D_0	$^{13}C_{rhizo}$ [3]	C_{soil} [4]
			kg ha^{-1}				‰	g kg^{-1}				mg	‰	mg	kg ha^{-1}
Wheat	WSL[1]	shoot labeled	5088	2209	74	30	383	377	298	287	38	31.3	–	–	–
		root unlabeled	1298	522	19	27	-28	251	360	301	88	–	-19.33	–	166
	WRL	shoot unlabeled	5083	2222	72	31	-28	385	292	278	45	–	–	–	–
		root labeled	1341	536	20	27	473	260	352	296	92	9.5	-16.73	2.9	166
Pea	PSL	shoot labeled	5065	2228	94	24	365	394	363	156	87	30.1	–	–	–
		root unlabeled	789	342	26	13	-30	342	275	240	143	–	-19.28	–	148
	PRL	shoot unlabeled	5059	2197	89	25	419	406	348	155	91	–	–	–	–
		root labeled	941	395	29	14	-30	346	272	251	130	6.0	-17.15	2.2	148
Vetch	VSL	shoot labeled	5081	2165	169	13	278	534	268	133	66	22.9	–	–	–
		root unlabeled	2089	819	65	13	-30	321	286	268	125	–	-19.48	–	240
	VRL	shoot unlabeled	5082	2175	158	14	-31	541	262	129	68	–	–	–	–
		root labeled	1796	717	55	13	330	326	297	258	120	10.0	-16.41	3.1	240

[1]WSL=wheat shoot labeled + wheat root unlabeled: WRL= wheat shoot unlabeled + wheat root labeled + soil: PSL pea shoot labeled + pea root unlabeled: PRL pea shoot unlabeled + pea root labeled + soil: VSL vetch shoot labeled + vetch root unlabeled: VRL= vetch shoot unlabeled + vetch root labeled + soil. ^{13}Cresidues[2]: amount of ^{13}C present in labeled roots and shoots on day 0.^{13}Crhizo[3]: amount of ^{13}C present in labeled roots treatments in the form of rhizodeposits per cylinder on day 0. $^{4}C_{soil}$: amount of C by rhizodeposition in soil prior to decomposition experiment, estimated after plant harvest on day 0 and assumed to be same in both labeled sub-plots and unlabeled sub-plots. Values are the mean of 3 replicates

Table 2 Summary of least significant difference (LSD) comparison results by the ANOVA test on the distribution of shoot and root (root+soil) derived C_{new} in different size soil aggregates at different sampling timings during the experiment

Depth	Size	Wheat				Pea				Vetch			
		Days				Days				Days			
cm	μm	0	60	180	365	0	60	180	365	0	60	180	365
0-5													
	>2000	A[1]	Aa[2]	Aa	Aa	B	Aa	Ba	Ba	C	Aa	ABa	BCa
	250-2000	B	Ba	Ba	Aa	Aa	Aa	Aa	Aa	B	Ba	Aa	Aa
	53-250	B	Bb	Bb	Aa	B	Ab	Bb	Aa	B	ABb	ABab	Aa
	<53	B	Ba	ABa	Aa	A	Aa	Aa	Aa	B	ABa	Ba	Aa
5-15													
	>2000	B	ABa	Aa	ABa	A	Aa	Aa	Aa	AB	Ab	ABc	Ba
	250-2000	A	ABa	Bb	Aa	A	ABa	Ba	ABa	A	ABa	Ba	Aa
	53-250	AB	Bb	Bb	Ba	A	ABb	Ab	ABa	A	Ab	Ab	Aa
	<53	AB	BCa	Cab	Ab	A	Aa	Ba	Ba	A	Aa	Ba	ABa
15-30													
	>2000	B	Ba	Aa	Ba	A	Aa	Aa	Aa	A	Aa	Aa	Aa
	250-2000	Aa	Ba	ABa	Aa	A	BCb	Cb	ABa	A	Bb	Bb	Aa
	53-250	Aa	Aa	Aa	Aa	A	ABa	Ba	ABa	A	Ab	Ab	Aa
	<53	A	Ab	Aab	Aa	A	ABa	BCa	Ca	A	Aa	Ab	Aab

[1]Different uppercase letters assigned within each aggregate size for each soil layer denote significant differences ($p < 0.05$) for root (root+soil) derived C_{new} whereas,
[2]lowercase letters denote significant differences ($p < 0.05$) for shoot derived C_{new}, between different sampling timings

Table 3 Temporal variation of aggregates distribution during the decomposition of wheat, pea and vetch ^{13}C labeled shoot- and root (root+soil) residues under no-till field conditions for the three soil layers (0-5, 5-15 and 15-30cm)

		Wheat				Pea				Vetch			
Depth	Size	Days				Days				Days			
cm	µm	0	60	180	365	0	60	180	365	0	60	180	365
						% soil dry weight							
0-5													
	>2000	69 ± 9.8	68 ± 2.5	58 ± 6.0	52 ± 3.6	62 ± 4.8	78 ± 6.8	65 ± 1.2	62 ± 1.2	71 ± 1.6	80 ± 3.3	65 ± 2.3	57 ± 3.3
	250-2000	18 ± 7.2	19 ± 1.3	23 ± 3.0	25 ± 2.8	23 ± 4.1	12 ± 5.0	21 ± 1.7	20 ± 1.1	16 ± 1.3	10 ± 1.8	21 ± 8.5	24 ± 1.9
	53-250	10 ± 3.3	10 ± 1.7	9 ± 3.3	19 ± 1.1	12 ± 0.5	7 ± 1.6	11 ± 1.4	15 ± 1.3	10 ± 1.4	7 ± 1.2	11 ± 3.2	15 ± 1.3
	<53	2 ± 0.85	2 ± 0.27	2 ± 0.53	4 ± 0.11	3 ± 0.28	2 ± 0.32	2 ± 0.06	3 ± 0.34	2 ± 0.18	2 ± 0.19	2 ± 0.54	2 ± 0.45
	LSD	15	3.8	8.7	5	7.3	10.0	0.73	1.39	2.95	4.67	17.7	4.73
5-15													
	>2000	14 ± 7.7	15 ± 1.0	10 ± 2.2	6 ± 2.5	13 ± 4.9	20 ± 5.9	10 ± 1.7	9 ± 1.7	20 ± 5.6	23 ± 4.3	14 ± 3.8	8 ± 1.2
	250-2000	56 ± 3.0	56 ± 1.3	57 ± 1.0	61 ± 3.8	60 ± 2.1	54 ± 3.2	64 ± 1.0	61 ± 1.1	56 ± 3.4	50 ± 3.6	57 ± 1.3	58 ± 1.0
	53-250	25 ± 5.3	24 ± 1.9	28 ± 3.0	27 ± 1.4	23 ± 2.7	22 ± 3.1	22 ± 1.4	26 ± 1.1	19 ± 2.3	22 ± 2.7	24 ± 3.5	30 ± 1.3
	<53	4 ± 0.37	4 ± 0.27	4 ± 0.31	5 ± 0.18	4 ± 0.39	4 ± 0.42	3 ± 0.22	4 ± 0.37	4 ± 0.42	4 ± 0.10	3 ± 0.38	4 ± 0.04
	LSD	11.3	2.9	4.5	5.5	6.9	8.6	2.9	2.5	10.9	7.2	6.2	2.4
15-30													
	>2000	5 ± 0.39	5 ± 2.8	5 ± 0.39	2 ± 0.45	7 ± 1.3	6 ± 2.2	7 ± 1.2	4 ± 1.1	7 ± 0.93	7 ± 3.5	7 ± 0.90	3 ± 0.42
	250-2000	56 ± 3.0	56 ± 4.0	55 ± 2.9	57 ± 2.2	60 ± 1.7	58 ± 3.2	58 ± 1.8	59 ± 1.4	59 ± 1.8	57 ± 2.1	58 ± 1.7	56 ± 2.5
	53-250	33 ± 1.9	30 ± 4.3	32 ± 3.2	36 ± 1.3	27 ± 0.82	30 ± 2.1	29 ± 1.5	32 ± 1.0	29 ± 1.9	30 ± 5.2	29 ± 2.0	37 ± 2.2
	<53	5 ± 0.27	5 ± 0.84	5 ± 0.13	5 ± 0.04	5 ± 0.18	8 ± 0.16	5 ± 0.17	5 ± 0.34	5 ± 0.39	5 ± 0.63	5 ± 0.40	4 ± 0.32
	LSD	5.1	7.5	5.0		2.71	5.1	2.7	2.4	3.1	7.7	3.2	3.9

Values followed by different letters in a column are significantly different between aggregates size classes of a given sampling time and soil layer at $P < 0.05$

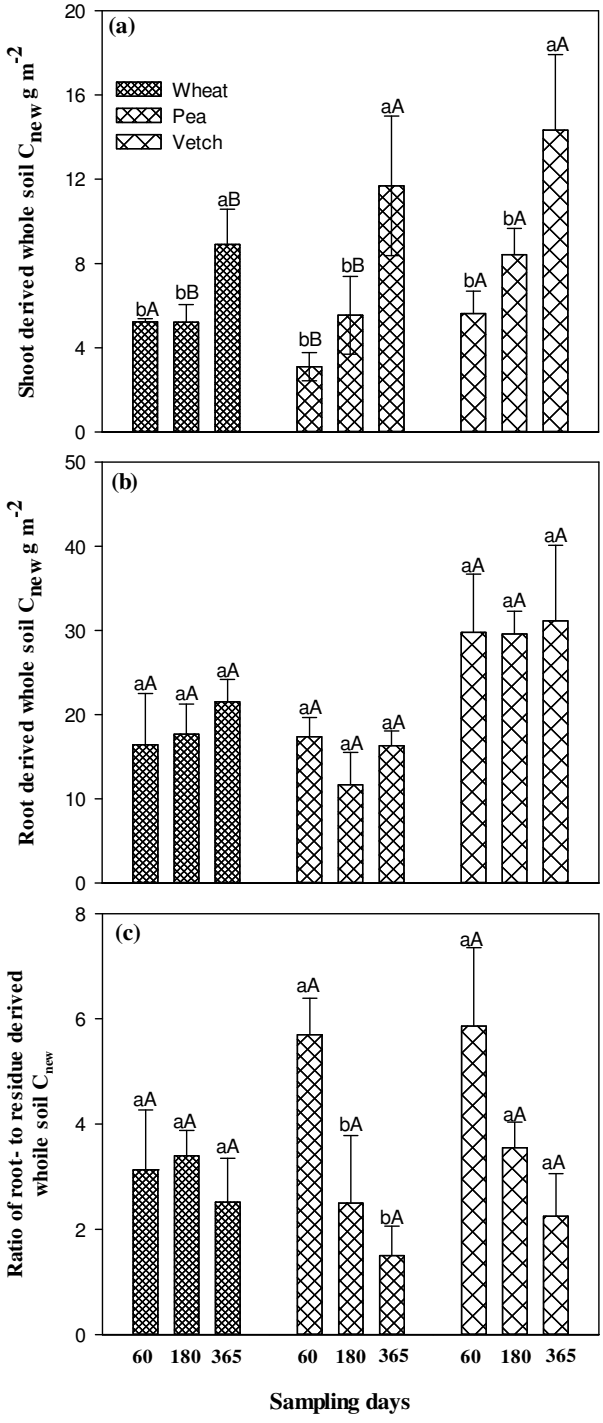

Fig. 2 C_{new} derived from shoot (a) , root residues (root+soil) (b) and relative contribution of root (root+soil) to shoot derived C_{new} for whole soil (0-30cm) at three sampling times (60, 180 and 365 days) (c). Vertical bars with different lower case letters represents significant differences between different sampling times for a crop and uppercase letters reflect significant differences between crops for the same sampling time at $P < 0.05$ level

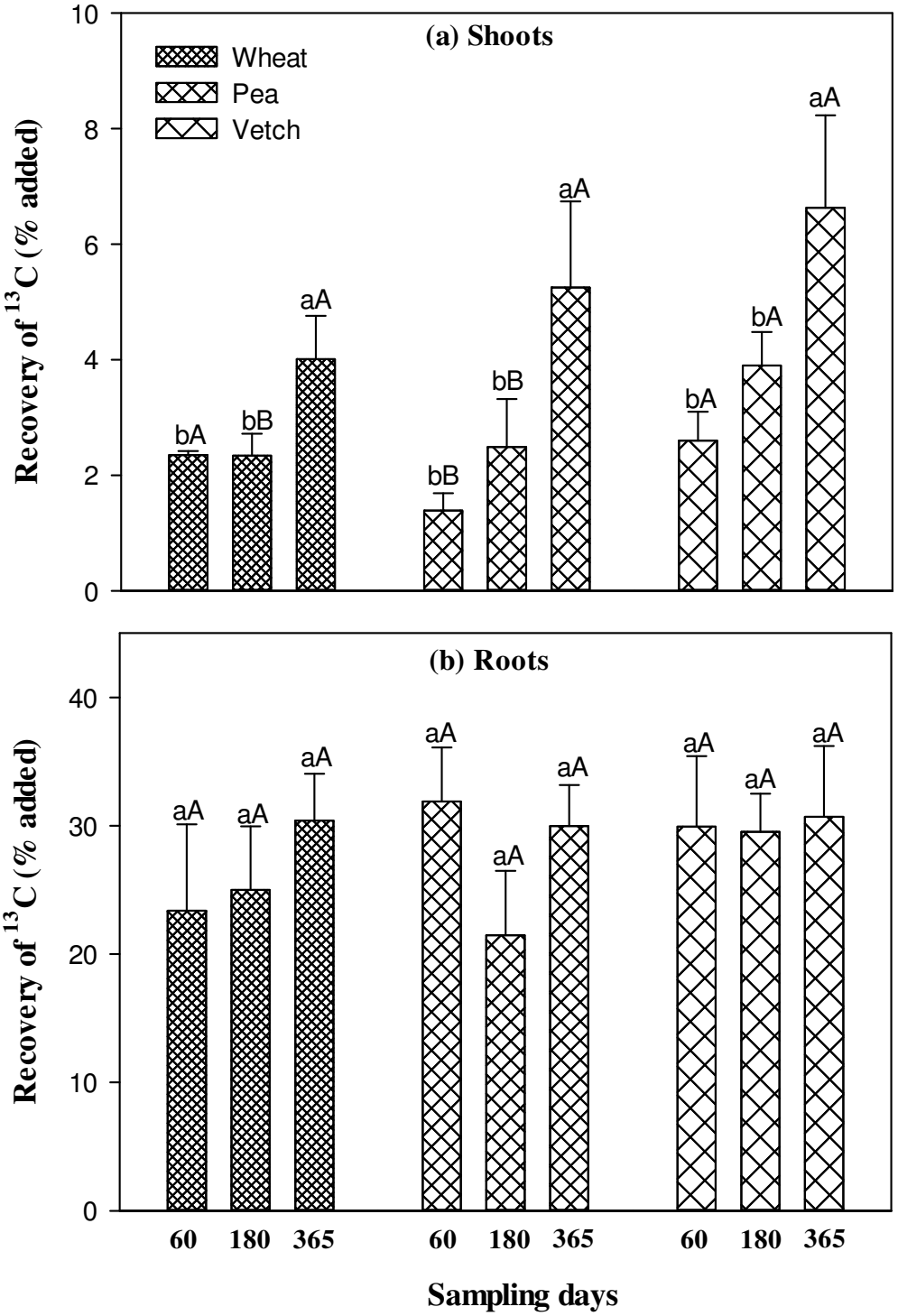

Fig. 3 Temporal variation in the recovery of shoot-derived ^{13}C (a) and root (root+soil) derived ^{13}C (b) for whole soil (0-30cm) in different size aggregates. Different capital and lowercase letters denote significant differences between crops on the same sampling time and between sampling times for the same crop at $P < 0.05$, respectively

Table 4 C derived from ^{13}C labeled wheat, pea and vetch shoot residues associated with aggregates size fractions in three soil depths (0-5, 5-15 and 15-30cm) during decomposition under no-till field conditions

Depth	Size	Wheat				Pea				Vetch			
		Days				Days				Days			
cm	µm	0	60	180	365	0	60	180	365	0	60	180	365
		C_{new} g m^{-2}											
0-5													
	>2000	0	3.71 ± 0.55	2.24 ± 1.02	2.32 ± 0.90	0	2.59 ± 1.52	2.24 ± 1.10	2.44 ± 0.70	0	4.44 ± 1.30	3.31 ± 0.92	2.67 ± 1.93
	250-2000	0	0.31 ± 0.04	0.50 ± 0.20	0.53 ± 0.35	0	0.19 ± 0.17	0.50 ± 0.15	0.63 ± 1.43	0	0.20 ± 0.09	0.74 ± 0.44	0.81 ± 1.46
	53-250	0	0.41 ± 0.07	0.62 ± 0.19	1.07 ± 0.05	0	0.34 ± 0.15	0.67 ± 0.05	1.76 ± 0.73	0	0.50 ± 0.08	0.99 ± 0.42	2.92 ± 1.81
	<53	0	0.18 ± 0.04	0.24 ± 0.06	0.26 ± 0.05	0	0.17 ± 0.02	0.28 ± 0.04	0.49 ± 0.28	0	0.19 ± 0.03	0.25 ± 0.10	0.30 ± 0.15
	LSD		0.55	1.11	1.03		1.61	1.15	1.09		ns	1.25	3.04
5-15													
	>2000	0	0.18 ± 0.05	0.09 ± 0.07	0.15 ± 0.13	0	0.35 ± 0.18	0.13 ± 0.09	0.28 ± 0.21	0	0.65 ± 0.43	0.21 ± 0.19	0.43 ± 0.36
	250-2000	0	0.63 ± 0.11	0.12 ± 0.05	0.95 ± 0.32	0	0.63 ± 0.13	0.27 ± 0.14	0.91 ± 0.67	0	0.80 ± 0.19	0.22 ± 0.06	3.03 ± 0.29
	53-250	0	0.30 ± 0.04	0.12 ± 0.15	0.88 ± 0.41	0	0.19 ± 0.09	0.11 ± 0.16	1.66 ± 0.77	0	0.38 ± 0.32	0.04 ± 0.05	1.84 ± 0.19
	<53	0	0.16 ± 0.06	0.03 ± 0.02	0.12 ± 0.08	0	0.15 ± 0.08	0.06 ± 0.03	0.18 ± 0.05	0	0.31 ± 0.15	0.07 ± 0.05	0.19 ± 0.18
	LSD		0.16	ns	0.57		0.23	ns	0.82		ns	ns	0.45
15-30													
	>2000	0	0.31 ± 0.24	0.24 ± 0.09	0.27 ± 0.06	0	0.27 ± 0.13	0.29 ± 0.08	0.30 ± 0.08	0	1.46 ± 0.21	0.47 ± 1.69	0.34 ± 0.02
	250-2000	0	0.67 ± 0.59	0.38 ± 0.21	1.23 ± 0.25	0	0.31 ± 0.35	0.25 ± 0.04	2.03 ± 0.13	0	0.43 ± 0.43	0.57 ± 1.44	3.47 ± 0.09
	53-250	0	0.52 ± 0.46	0.20 ± 0.22	0.94 ± 0.64	0	0.22 ± 0.10	0.12 ± 0.03	1.10 ± 0.87	0	0.59 ± 0.39	0.22 ± 0.82	1.76 ± 0.34
	<53	0	0.35 ± 0.21	0.07 ± 0.05	0.41 ± 0.06	0	0.19 ± 0.15	0.14 ± 0.11	0.42 ± 0.10	0	0.29 ± 0.14	0.10 ± 0.23	0.18 ± 0.02
	LSD		ns	0.35	ns		ns	ns	ns		ns	ns	0.36

Values followed by different letters in a column are significantly different between aggregates size classes of a given sampling time and soil layer at $P < 0.05$

Table 5 C derived from ^{13}C labeled wheat, pea and vetch root residues (roots+soil) associated with aggregates size fractions in three soil depths (0-5, 5-15 and 15-30cm) during decomposition under no-till field conditions

		Wheat				Pea				Vetch			
Depth	Size	Days				Days				Days			
cm	µm	0	60	180	365	0	60	180	365	0	60	180	365
						C_{new} g m^{-2}							
0-5													
	>2000	8.42 ± 1.97	11.2 ± 6.35	8.41 ± 0.10	9.09 ± 2.57	4.96 ± 0.99	10.7 ± 1.88	6.37 ± 0.31	6.45 ± 0.93	7.08 ± 1.42	17.5 ± 1.28	14.4 ± 3.20	10.1 ± 0.84
	250-2000	0.54 ± 0.21	0.77 ± 0.16	0.91 ± 1.10	2.05 ± 0.23	0.65 ± 0.12	0.96 ± 0.60	1.00 ± 0.31	1.35 ± 0.25	0.66 ± 0.14	0.96 ± 0.31	1.79 ± 0.42	2.46 ± 0.49
	53-250	0.54 ± 0.16	0.63 ± 0.08	1.70 ± 0.20	1.34 ± 0.29	0.66 ± 0.24	1.29 ± 0.11	0.56 ± 0.03	1.18 ± 0.30	0.70 ± 0.15	1.29 ± 0.57	1.34 ± 0.21	1.96 ± 0.22
	<53	0.22 ± 0.07	0.20 ± 0.02	0.28 ± 0.06	0.37 ± 0.04	0.29 ± 0.12	0.44 ± 0.03	0.29 ± 0.03	0.35 ± 0.09	0.31 ± 0.01	0.44 ± 0.08	0.36 ± 0.07	0.56 ± 0.13
	LSD	1.90	2.58	0.21	2.67	0.88	2.17	0.49	1.09	1.46	1.49	3.41	1.02
5-15													
	>2000	0.81 ± 0.41	1.20 ± 0.24	2.44 ± 0.62	1.22 ± 0.96	1.49 ± 0.90	1.79 ± 0.70	0.49 ± 0.70	1.55 ± 1.07	3.52 ± 0.61	5.37 ± 2.00	3.22 ± 1.69	2.56 ± 1.98
	250-2000	2.07 ± 0.99	1.38 ± 0.78	0.85 ± 0.44	2.89 ± 0.29	2.53 ±0.59	0.93 ± 0.79	0.23 ± 0.35	1.90 ± 0.66	5.08 ± 0.68	3.84 ± 0.28	2.73 ± 1.44	5.00 ± 0.70
	53-250	0.90 ± 0.65	0.51 ± 0.32	0.11 ± 0.11	1.07 ± 0.15	0.61 ± 0.11	0.54 ± 0.27	0.19 ± 0.28	0.50 ± 0.26	1.31 ± 0.19	1.01 ± 0.30	0.95 ± 0.82	1.88 ± 0.79
	<53	0.37 ± 0.13	0.22 ± 0.09	0.14 ± 0.04	0.38 ± 0.09	0.38 ± 0.02	0.28 ± 0.11	0.06 ± 0.03	0.12 ± 0.08	0.65 ± 0.08	0.65 ± 0.16	0.22 ± 0.23	0.50 ± 0.22
	LSD	1.03	0.84	0.82	1.06	1.22	0.84	Ns	ns	0.99	2.17	1.70	1.62
15-30													
	>2000	0.69 ± 0.10	0.71 ± 0.45	1.94 ± 0.11	0.50 ± 0.37	1.58 ± 0.61	1.32 ± 0.24	1.88 ± 0.96	0.99 ± 0.26	1.49 ± 0.62	1.47 ± 1.65	1.19 ± 0.12	0.94 ± 0.26
	250-2000	3.11 ± 0.80	1.28 ± 0.45	2.25 ± 0.80	3.30 ± 0.54	4.21 ± 0.78	2.94 ± 0.43	2.04 ± 0.63	3.22 ± 0.89	4.70 ±1.28	0.91 ± 1.06	1.18 ± 1.00	4.00 ± 1.54
	53-250	0.96 ± 0.30	0.81 ± 0.60	1.23 ± 0.27	1.20 ± 0.58	1.25 ± 0.36	0.97 ± 0.43	0.27 ± 0.08	0.54 ± 0.41	1.52 ± 0.30	0.98 ± 0.56	1.16 ± 0.66	1.62 ± 0.39
	<53	0.55 ± 0.09	0.55 ± 0.14	0.55 ± 0.02	0.47 ± 0.09	0.69 ± 0.06	0.57 ± 0.06	0.27 ± 0.05	0.23 ± 0.31	0.65 ± 0.09	0.68 ± 0.21	0.64 ± 0.20	0.42 ± 0.17
	LSD	0.79	ns	0.91	0.78	0.87	0.73	0.95	0.97	1.15	ns	ns	1.53

Values followed by different letters in a column are significantly different between aggregates size classes of a given sampling time and soil layer at $P < 0.05$

5 GENERAL DISCUSSION

Plant residue decomposition is a complex process strongly affected by biotic (quality of residue and soil microbial composition) and abiotic factors (moisture and temperature). Though, several mechanisms are well known, the mineralization and stabilization of carbon (C) derived from shoot and roots of crop plants under no-till soil conditions is still poorly known. A broader knowledge of the plants shoot and root C turnover in soil can help to increase our knowledge of global C-cycling with particular regard to climate relevant CO_2. It is therefore important to distinguish between these two component of plants residues because they represent different C mineralization and stabilization pathways which are likely to contribute to soil in different ways and amounts. The general objective of the work presented in this thesis was to estimate the short term C mineralization dynamics and the fate of the shoot and root derived C of three crops in soil under no-till field conditions. The main contributions of the research contained within this thesis have, therefore, been to the: (1) isotopic labeling of plants under field conditions for subsequent C mineralization and stabilization studies, (2) mineralization of above- and below-ground residue for a more holistic picture of no-till system C cycling, and (3) to the stabilization of shoot and root derived C in different aggregate size classes under no-till conditions.

The experimental approach i.e. pulse labeling of plants under field conditions was chosen: 1) since it is not possible to track shoot and root residues C decomposing simultaneously, until they have distinct isotopic ratios, and 2) to avoid possible bias introduced in the decomposition dynamics of the residues particularly roots due to collection, washing and drying under controlled conditions compared to intact roots under field conditions.

^{13}C labeling

Crop plants were labeled under field conditions by using labeling chambers (Artigo 1). The results showed that plant biomass production and chemical composition was not affected either by pulse labeling or by growing plants inside soil cylinders. This is a necessary prerequisite for studying simultaneous decomposition of shoot and root residues, since “paired” treatments were made by the exchange of the labeled residues with unlabeled

residues to mimic actual field conditions. A relatively higher biomass production of the crop plants compared to that reported earlier (BOODY et al., 2010) for the same crops under field conditions was due to better managements of the plants. Although, the complete homogeneity in the distribution of ^{13}C label among plant parts and chemical fractions was not achieved, the heterogeneity was less in chemical fractions compared to whole plant parts. Many authors despite the heterogeneity of ^{13}C label within the plant parts and chemicals fractions followed shoot and root derived C through several soil pools (PUGET; DRINKWATER, 2001; WILLIAMS et al., 2006; KONG; SIX, 2010). The level of ^{13}C enrichment in my experiment was much greater in comparison to ^{13}C enrichments levels of plants parts either under controlled conditions (i.e. SANGSTER et al., 2010) or field conditions (PUGET; DRINKWATER, 2001; KONG; SIX, 2010). I assumed that the frequency and span of labeling was responsible for the increased ^{13}C enrichment of plants in my experiment. Another possible reason is the re-fixation of overnight-respired $^{13}CO_2$, since plants were enclosed inside labeling chambers after each labeling event till following morning. Therefore, I believe that that labeled plant residues can be used for subsequent tracking of shoot or root derived C in various soil C pools. The recovery of ^{13}C applied was though low but it was within the range reported in other studies (i.e. WICHERN et al., 2007). I believe that much of the applied ^{13}C was lost during the growth of the plants through shoot and root respiration and the mineralization of the ^{13}C in soil (rhizodeposits). OSTLE et al. (2000) found that 76.4 % and 61.65 % of the added ^{13}C was respired within 24 hours from shoots and roots respectively. Thus it could be expected that some c.a. 60 % of all the added ^{13}C in this experiment has been lost trough plant respiration and due to the mineralization of ^{13}C in soil (rhizodeposits).

C mineralization

The second objective of this study was to investigate the short term C mineralization dynamics of shoots and intact roots residues of three winter crops under no-till field conditions (Artigo 2). I used “paired” treatments (^{13}C labeled shoot + unlabeled root and unlabeled shoot + ^{13}C labeled root+soil) and static chamber technique to capture total CO_2 ($^{13}CO_2$ + $^{12}CO_2$) evolved during the simultaneous decomposition of labeled and unlabeled residues and native soil organic matter (SOM). My results indicated that total C-CO_2 evolved from “paired” treatments were similar in kinetics and C mineralization rates which was expected since the labeling of plants did not affect C contents and chemical composition of

the plants (Artigo 1). The similar rates of C mineralization between "paired" treatments was a necessary prerequisite in the first place to calculate the actual amount of C mineralizing from shoot, roots (root+soil) and native SOM. The data indicated that in undisturbed soil, a large proportion of root-derived C was evolved as CO_2 compared to a smaller proportion of surface shoot-derived C-CO_2 during decomposition. This result contradicts to the universal pattern of slower decomposition of root residues compared to shoot residues (FRESCHET et al., 2013) often attributed to higher intrinsic chemical recalcitrance of the roots residues (RASSE et al., 2005). In fact, slower decomposition of root *vs* shoot implies that, all conditions being equal, root residues decomposed at a slower rate in soil than their shoots. Measuring this effect implies that root and shoot residues are subjected to equal conditions in soils (RASSE et al., 2005), which was not the case in my experiment. Laboratory incubation studies meet this requirement, while field studies compound multiple effects on crop residue decomposition, such as location, moisture, temperature and contact with soil, especially under no-till conditions. That's why my results appeared to contradict most of the studies conducted in laboratory conditions (GALE; CAMBARDELLA, 2000; LU et al., 2003; ABIVEN et al., 2005). My results also did not agree with the findings of PUGET; DRINKWATER, (2001) and WILLIAMS et al. (2006) under field conditions because the shoot residues were incorporated with roots which confirms the proposition of RASSE et al. (2005).

I believe that apart from the chemical composition differences among shoot and root residues, the decomposition was much influenced by the position of the residues. The belowground abiotic conditions and intimacy of contact between soil and roots lead to the higher decomposition of roots compared to shoot residues. COPPENS et al. (2006) estimated that only 10 % of the oilseed rape residues left at soil surface was in direct contact with the soil, which was not the case for roots. BUYANOVSKY; WAGNER (1987) found increased mineralization of intact roots (53 % of added C) compared to surface shoot residues (49 % of added C) after 1 year. Similarly, several studies reported that decomposition rates for many different crop residue types were consistently higher for residues that are buried compared to those placed on the soil surface (SUMMERELL; BURGESS, 1989; SOON; ARSHAD, 2002; COPPENS et al., 2006). An undesirable character associated with labeling and studying C mineralization in undisturbed soil is the translocation of rhizodeposits from root to soil far ahead of the release of root derived C. Therefore, a significant contribution of this labeled pool to the evolved ^{13}C-CO_2 in the LR (roots+soil) treatments cannot be excluded but could not be quantified separately. This suggests that the mineralization rate of the "roots" ^{13}C could be overestimated by including initial plant-derived ^{13}C in the soil. To avoid this, I estimated

rhizodeposits after plant harvest and included them in calculations to reduce possible bias in the mineralization of root C. Additionally, the high root C mineralization was also confirmed by the low remaining roots C pool after 180 days. Overall there was little effect of the types of crops on the total C mineralized in 180 days since most of the evolved C-CO_2 was coming from the SOM. For all the three crops, shoots and roots + soil derived C-CO_2 kinetics followed the "classical" two phases of mineralization i.e. an initial phase of fast mineralization followed by a phase of slower mineralization.

I also observed extra mineralization of native SOM in the early stage of decomposition in residue amended treatments compared to control soil which corresponded to about 0.23 % (W and P) and 0.09 % (V) of total soil C in the top 30 cm soil. I hypothesized that the extra mineralization of native SOM could be due to: 1) the acceleration of native soil organic C (SOC) decomposition due to priming effect, i.e. the supply of fresh organic C to soil microorganisms increased the degradation of native SOM by the soil microorganisms (NOTTINGHAM et al., 2009; GARCIA-PAUSAS; PATERSON, 2011; GUENET et al., 2012), and 2) the extra mineralization could also result from more favorable soil conditions for SOM mineralization in the residue-amended cylinders compared to the control cylinders. Since the two processes are not mutually exclusive, but considering that the main difference occurred shortly after start of plant residues decomposition, I hypothesized that extra C mineralization could be due to a real priming effect.

The ^{13}C balance after 180 days was different between LS (shoot labeled + root unlabeled) and LR (shoot unlabeled + root labeled + soil). The recovery was less than 100 % in LS treatments while it was above 100 % in LR treatments. Several studies involving labeled residues reported a lack of recovery and this was attributed to losses in C-CO_2 trapping during the mineralization studies (PUGET; DRINKWATER, 2001). Besides the losses of ^{13}C-CO_2, manual application of water after cover removing to compensate for rain occurring during the closing of the cylinders, could lead to extra ^{13}C-CO_2 emissions particularly with shoots mulches at the soil surface, therefore not captured by NaOH trap. The peaks of $^{13}CO_2$ emissions derived from shoots-C, observed especially after rainfall events, support this hypothesis. On the contrary, I believe that higher recovery in LR treatments was could be due to the $^{13}CO_2$ emissions coming from the mineralization of roots+soil below 30 cm soil depth i.e. outside the volume of soil sampled in cylinders. Since, some roots were observed below 30 cm soil depth at day 0 when removing the initial cylinders, but it was impossible to remove quantitatively roots below 30 cm depth.

C stabilization

The third objective of this study was to track the destination of C_{new} derived from shoot and root residues of the three winter crops in aggregates of different sizes under no-till field conditions (Artigo 3). My results showed that the distribution of shoot and root derived C followed a similar pattern during simultaneous decomposition at different positions under no-till field conditions and was in the order of Ma > Mi > silt +clay fractions in all three soil layers. LMa were the most prominent C storage location for both shoot and root residues in 0-5 cm soil layer, while SMa in lower layers (5-15 and 15-30 cm). On an average of the crops and soil layers, Ma (LMa + SMa) retained up to 64 % and 80 % of the C_{new} derived from shoot and root residues, respectively after 365 days. The higher retention of C_{new} in the macroaggregates was possibly due to the formation of more particulate organic C in the Ma than in the Mi. My results agree with the findings of OLCHIN et al. (2008) from a 1 yr long *in situ* decomposition experiment with ^{13}C labeled wheat shoot residues under no-till conditions. They found a higher percentage of wheat shoot-derived C in Ma. With the time C_{new} in LMa decreased whereas C_{new} in SMa and Mi increased significantly, with few exception for root residues in 5-15 and 15-30 cm layers. ANGERS et al. (1997) observed that when labeled wheat straw was incorporated into the soil, the concentration of newly added C in Ma was initially high and then declined, but the concentration of C_{new} in Mi increased with time. The lowest C_{new} was found in the silt + clay fraction. This fraction contained no sand particles but it might be that the low C_{new} in this fraction was influenced by low clay contents and the accumulation of free silt particles with low or no binding capacity of SOC (e.g. small quartz grains) (JOHN et al., 2005).

The amount of C_{new} derived from shoot and root residues was different. Although more than 2 (vetch), 3 (wheat) and 4 (pea) times more shoot C than root C (root+soil) was added, only 8.9 (wheat), 11.6 (pea) and 14.3 (vetch) g C_{new} m^{-2} of shoot C *vs* 23.8 (wheat), 18.3 (pea) and 32.0 (vetch) g C_{new} m^{-2} of root C (root+soil) remained in the whole soil after 1 yr. My results suggest that the differences in the decomposing conditions and stabilization mechanisms explains the differences in amounts of shoot *vs* root derived C_{new} in soil. When the shoot residues were left on the soil surface, some of the residue C enters in the soil by diffusion at the soil-residue interface near the soil surface (GAILLARD et al., 1999) and by convective transport after rain (COPPENS et al., 2006). Consequently, C originating from surface shoot residues would lack the physical protection that root C has (MENICHETTI et al., 2015). In contrast, root residues are in the soil and higher retention of root derived C_{new}

in soil is due to their close proximity to the soil matrix. Another explanation of the higher root derived C_{new} in soil is the stabilization and protection of the labile root C during the growth and early stages of decomposition. SCHMIDT et al. (2011) proposed that SOM persistence in soil is more due to environmental factors that reduce decomposition than to intrinsic properties of the organic matter itself. Additionally, the variability in C_{new} of the lower soil layers (5-15 and 15-30 cm) could be due to the leaching of dissolved organic C from upper soil layer which complicated my results. It seems likely, that a significant amount of C could be transported deeper in the soil through leaching in our experimental conditions, especially after every rainfall events.

Since it is known that high quality residues decomposes fast (i.e., labile) and 'low-quality' residues decomposes slowly (i.e., recalcitrant). Earlier it was believed that low quality residues decompose slowly and contribute more to SOM formation (RASSE et al., 2006). However, recently COTRUFO et al. (2013) proposed that high quality residues produce more microbial residues than low quality residues which provides more SOM stabilization opportunities. My results in the present experiment support this hypothesis when I compared roots and shoots separately among crops. Besides the less variability in chemical composition, I observed that vetch shoot and root residues which were the high quality residues in my experiment contributed more C_{new} compared to pea and wheat after 1 yr, though not different significantly. On the other hand, when roots were compared with shoots, my results does not corroborate with the hypothesis of COTRUFO et al. (2013) in terms of quality of residues, since roots are known as low quality residues. However, my results support the speculation of COTRUFO et al. (2013) in terms of microbial substrate use. In my experiment, roots decomposed faster than shoot residues which means that root residues produced more microbial residues (high microbail susbstrate use) than shoot residues and hence more C to SOM. CASTELLANO et al. (2015) also proposed that plant residues that produce more microbial residues result in more organic matter that can be physico-chemically stabilized. Finally, my results show that when residues of different chemical quality are decomposing in different positions, abiotic factors associated with location prevail on quality in terms of C mineralization and stabilization in soil.

Conclusions

The repeat pulse labeling method holds great promise for labeling plants under field conditions and heterogeneity of ^{13}C label within plant parts could be minimized by more

frequent labeling sessions; may be twice a week. Though, it will increase the cost of labeling but it will be far less than the cost of continuous labeling covering the entire growth cycle of the plants. Based on the data obtained in the experiments, it is concluded the decomposition of intact roots belowground was fast and greater than shoot residues decomposing aboveground. This finding highlight the importance of my study in revealing the role of a realistic experimental system (actual no-till field conditions) on residue decomposition, especially for intact roots, whereas high lignin contents are often used as justification for the slow decomposition of roots in laboratory experiments. Roots contributed relatively more C_{new} than shoot residues. The greater association of root and shoot derived C_{new} with Ma highlights the importance of Ma in C sequesteration under no-till field conditins. My results show that quality of residues impact residues decomposition and C stabilization in soil, only when residues are decomposing in similar environmental conditions i.e. high quality residues decompose and contribute more C in soil. In contrast, location of the residues prevail on quality, through its influence on decomposition which in turn impact C stabilization in soil i.e. greater decomposition will provide more microbial residues and hence more stabilized C in soil. In conclusion, my study promoted the understanding of the fate of root *vs* shoot C in soil under no-till field conditions.and has important implications for developing management strategies e.g. selection of crops to be used in crop rotations to optimize C sequestration in soil.

6 PERSPECTIVES AND FUTURE RESEARCH

The work presented in this thesis described the processes of C cycling of shoot and root residues decomposing simultaneously under no-till field conditions. In fact, the combined use of ^{13}C labeled shoot and root residues, periodic sampling, and physical fractionation made this study unique in tracking the progressive changes in mineralization and the distribution of shoot and root derived C in various aggregate size classes. These results should direct our attention to the unseen and often ignored contributions of roots and root exudates to soil organic matter for future research. Since Brazil is a big country, comprised of several ecosystems, future research should consider the followings:

1) Crops with different root systems should be included in future research to explore their potential in C sequestration under no-till conditions.

2) Differences in crop residue quality should be explored further under different climatic conditions, growing seasons and soil types with respect to their effect on C mineralization and stabilization.

3) Identification and composition of soil microbial communities associated with surface shoot and belowground root residues should also be considered.

4) The sampling depth and the measurement of leached/dissolved organic C should also be considered in future experiments.

5) Finally, long term studies should be made with similar techniques to determine how long it takes for fresh residues to reach a stable state into the SOM.

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APÊNDICES

Appendix 1. Temporal variation in carbon concentration (%) of different sizes of soil aggregates (%) under wheat, pea and vetch shoot and root residues (roots+soil) at three soil depths (0-5, 5-15 and 15-30cm) under no-till field conditions (means ± SE).

Depth	Size	Wheat				Pea				Vetch			
		Days				Days				Days			
cm	µm	0	60	180	365	0	60	180	365	0	60	180	365
							%						
0-5													
	>2000	1.43 ± 0.20	1.37 ± 0.09	1.33 ± 0.15	1.51 ± 0.15	1.25 ± 0.26	1.39 ± 0.04	1.27 ± 0.11	1.54 ± 0.11	1.19 ± 0.19	1.29 ± 0.06	1.27 ± .015	1.41 ± 0.15
	250-2000	0.82 ± 0.04	0.86 ± 0.04	0.81 ± 0.06	0.89 ± 0.06	0.75 ± 0.09	0.80 ± 0.05	0.76 ± 0.04	1.00 ± 0.04	0.75 ± 0.11	0.74 ± 0.10	0.76 ± 0.07	0.91 ± 0.07
	53-250	0.89 ± 0.08	0.90 ± 0.07	0.84 ± 0.10	0.79 ± 0.10	0.85 ± 0.22	0.88 ± 0.05	0.80 ± 0.04	0.92 ± 0.04	0.82 ± 0.07	0.96 ± 0.12	0.83 ± 0.15	0.93 ± 0.15
	<53	1.56 ± 0.24	1.71 ± 0.26	1.60 ± 0.29	1.51 ± 0.29	1.50 ± 0.23	1.88 ± 0.16	1.63 ± 0.01	1.92 ± 0.01	1.63 ± 0.15	1.73 ± 0.10	1.83 ± 0.14	2.19 ± 0.14
5-15													
	>2000	0.89 ± 0.18	0.80 ± 0.10	0.89 ± 0.14	0.87 ± 0.14	0.72 ± 0.04	0.77 ± 0.04	0.87 ± 0.24	0.78 ± 0.24	0.73 ± 0.05	0.75 ± 0.02	0.71 ± 0.03	0.82 ± 0.03
	250-2000	0.59 ± 0.05	0.62 ± 0.01	0.60 ± 0.02	0.59 ± 0.02	0.60 ±0.04	0.59 ± 0.04	0.57 ± 0.02	0.59 ± 0.02	0.57 ± 0.04	0.58 ± 0.07	0.57 ± 0.01	0.55 ± 0.01
	53-250	0.55 ± 0.03	0.53 ± 0.02	0.53 ± 0.02	0.51 ± 0.02	0.51 ± 0.02	0.51 ± 0.01	0.49 ± 0.02	0.53 ± 0.02	0.51 ± 0.03	0.52 ± 0.02	0.51 ± 0.01	0.51 ± 0.01
	<53	1.09 ± 0.07	1.07 ± 0.06	1.13 ± 0.06	1.17 ± 0.06	1.14 ± 0.02	1.09 ± 0.04	1.11 ± 0.05	1.31 ± 0.06	1.09 ± 0.01	1.10 ± 0.04	1.19 ± 0.01	1.37 ± 0.01
15-30													
	>2000	0.68 ± 0.03	0.71 ± 0.06	0.67 ± 0.02	0.82 ± 0.02	0.78 ± 0.27	0.67 ± 0.02	0.78 ± 0.11	0.78 ± 0.11	0.62 ± 0.04	0.67 ± 0.03	0.66 ± 0.06	0.72 ± 0.06
	250-2000	0.54 ± 0.03	0.59 ± 0.04	0.55 ± 0.02	0.51 ± 0.03	0.59 ± 0.06	0.58 ± 0.02	0.56 ± 0.02	0.57 ± 0.02	0.54 ± 0.05	0.57 ± 0.01	0.53 ± 0.03	0.52 ± 0.03
	53-250	0.52 ± 0.01	0.55 ± 0.02	0.52 ± 0.02	0.54 ± 0.07	0.59 ± 0.14	0.55 ± 0.03	0.52 ± 0.02	0.57 ± 0.02	0.52 ± 0.04	0.52 ± 0.02	0.52 ± 0.02	0.52 ± 0.02
	<53	1.03 ± 0.04	1.10 ± 0.07	1.07 ± 0.07	1.11 ± 0.03	1.18 ± 0.17	1.14 ± 0.09	1.07 ± 0.02	1.19 ± 0.02	1.00 ± 0.14	1.10 ± 0.08	1.15 ± 0.03	1.29 ± 0.03

Appendix 2. Temporal variation in the δ ^{13}C (‰) values of of different aggregate size classes under surface wheat, pea and vetch labeled shoot residues at three soil depths (0-5, 5-15 and 15-30cm) under no-till field conditions (means ± SE).

Depth	Size	Wheat				Pea				Vetch			
		Days				Days				Days			
cm	µm	0	60	180	365	0	60	180	365	0	60	180	365
							‰						
0-5													
	>2000	-23.1 ± 0.61	-20.8 ± 0.28	-21.4 ± 0.84	-21.4 ± 0.73	-23.1 ± 0.61	-21.8 ± 0.72	-21.5 ± 0.81	-21.7 ± 0.25	-23.1 ± 0.61	-21.2 ± 0.52	-21.3 ± 0.18	-21.7 ± 0.67
	250-2000	-20.9 ± 0.29	-19.9 ± 0.24	-19.6 ± 0.34	-19.5 ± 1.05	-20.9 ± 0.29	-20.0 ± 0.51	-19.4 ± 0.41	-19.3 ± 1.14	-20.9 ± 0.29	-19.8 ± 0.65	-19.0 ± 0.69	-19.4 ± 0.88
	53-250	-22.4 ± 0.49	-19.9 ± 0.37	-19.4 ± 0.12	-18.3 ± 0.16	-22.4 ± 0.49	-19.3 ± 1.29	-17.4 ± 0.59	-15.6 ± 2.00	-22.4 ± 0.49	-19.3 ± 0.88	-17.8 ± 1.33	-13.1 ± 6.12
	<53	-21.8 ± 0.63	-18.9 ± 0.07	-18.2 ± 0.18	-19.1 ± 0.75	-21.8 ± 0.63	-18.9 ± 0.38	-17.3 ± 0.61	-16.8 ± 2.69	-21.8 ± 0.63	-18.9 ± 0.79	-19.0 ± 0.68	-19.4 ± 1.22
5-15													
	>2000	-20.5 ± 1.48	-20.1 ± 0.08	-20.3 ± 0.14	-19.9 ± 0.30	-20.5 ± 1.48	-20.0 ± 0.11	-20.1 ± 0.24	-19.6 ± 0.81	-20.5 ± 1.48	-19.9 ± 0.31	-20.1 ± 0.21	-19.4 ± 0.91
	250-2000	-19.2 ± 0.54	-18.8 ± 0.07	-19.1 ± 0.03	-18.6 ± 0.17	-19.2 ± 0.54	-18.7 ± 0.05	-19.0 ± 0.07	-18.6 ± 0.42	-19.2 ± 0.54	-18.7 ± 0.06	-19.0 ± 0.03	-17.6 ± 0.10
	53-250	-19.0 ± 0.31	-18.5 ± 0.10	-18.7 ± 0.22	-17.7 ± 0.61	-19.0 ± 0.31	-18.7 ± 0.18	-18.8 ± 0.26	-16.5 ± 1.23	-19.0 ± 0.31	-18.5 ± 0.46	-19.0 ± 0.06	-17.1 ± 0.26
	<53	-19.3 ± 0.25	-18.5 ± 0.27	-19.2 ± 0.07	-18.4 ± 0.37	-19.3 ± 0.25	-18.5 ± 0.56	-19.0 ± 0.16	-18.6 ± 0.23	-19.3 ± 0.25	-18.2 ± 0.52	-19.0 ± 0.19	-18.7 ± 0.51
15-30													
	>2000	-20.5 ± 0.28	-19.1 ± 0.48	-19.3 ± 0.51	-16.8 ± 0.26	-20.5 ± 0.28	-19.4 ± 0.19	-19.6 ± 0.10	-18.8 ± 0.64	-20.5 ± 0.28	-19.3 ± 0.34	-19.1 ± 0.73	-18.1 ± 0.10
	250-2000	-18.4 ± 0.17	-18.0 ± 0.31	-18.2 ± 0.12	-17.7 ± 0.06	-18.4 ± 0.17	-18.3 ± 0.18	-18.3 ± 0.02	-17.4 ± 0.07	-18.4 ± 0.17	-18.2 ± 0.18	-18.2 ± 0.11	-16.8 ± 0.06
	53-250	-17.9 ± 0.62	-17.3 ± 0.41	-17.7 ± 0.23	-17.0 ± 0.59	-17.9 ± 0.62	-17.7 ± 0.10	-17.8 ± 0.04	-16.8 ± 0.95	-17.9 ± 0.62	-17.4 ± 0.26	-17.7 ± 0.09	-16.7 ± 0.14
	<53	-18.5 ± 0.45	-17.5 ± 0.53	-18.3 ± 0.15	-17.4 ± 0.11	-18.5 ± 0.45	-18.0 ± 0.44	-18.1 ± 0.34	-17.2 ± 0.35	-18.5 ± 0.45	-17.8 ± 0.40	-18.3± 0.20	-18.1 ± 0.03

Appendix 3. Temporal variation in the δ ^{13}C (‰) values of of different aggregate size classes under intact wheat, pea and vetch labeled roots (root+soil) at three soil depths (0-5, 5-15 and 15-30cm) under no-till field conditions (means ± SE).

Depth	Size	Wheat				Pea				Vetch			
		Days				Days				Days			
cm	µm	0	60	180	365	0	60	180	365	0	60	180	365
							‰						
0-5													
	>2000	-16.6 ± 2.65	-14.2 ± 5.43	-15.2 ± 0.82	-14.7 ± 2.34	-19.1 ± 0.66	-17.1 ± 0.73	-18.4 ± 0.48	-18.9 ± 0.79	-18.2 ± 1.54	-13.6 ± 0.52	-13.4 ± 1.04	-15.6 ± 1.51
	250-2000	-18.3 ± 0.17	-17.6 ± 0.92	-17.6 ± 0.53	-14.4 ± 1.05	-18.6 ± 0.25	-15.4 ± 1.74	-17.5 ± 0.97	-16.9 ± 0.79	-18.0 ± 0.28	-14.1 ± 0.65	-14.2 ± 0.72	-16.6 ± 1.75
	53-250	-17.9 ± 0.26	-17.2 ± 1.92	-18.0 ± 0.61	-15.8 ± 0.16	-18.4 ± 0.40	-16.3 ± 0.80	-17.7 ± 0.20	-17.2 ± 0.68	-17.4 ± 0.47	-12.0 ± 0.88	-14.1 ± 0.64	-14.4 ± 1.38
	<53	-17.3 ± 0.50	-17.6 ± 1.10	-16.5 ± 0.28	-17.0 ± 0.75	-17.5 ± 1.22	-16.5 ± 1.11	-16.1 ± 0.62	-17.8 ± 0.67	-16.7 ± 0.71	-13.5 ± 0.79	-16.5 ± 0.20	-16.0 ± 1.52
5-15													
	>2000	-18.6 ± 1.72	-17.7 ± 0.62	-13.0 ± 1.24	-15.0 ± 3.33	-16.9 ± 1.10	-17.7 ± 0.24	-18.9 ± 2.08	-14.8 ± 4.09	-15.7 ± 1.70	-14.9 ± 1.03	-13.7 ± 4.33	-12.7 ± 5.75
	250-2000	-17.4 ± 0.87	-18.0 ± 0.65	-18.4 ± 0.38	-17.0 ± 0.12	-17.4 ± 0.24	-18.4 ± 0.67	-18.8 ± 0.24	-17.9 ± 0.50	-16.1 ± 0.16	-16.7 ± 0.46	-17.6 ± 0.76	-16.2 ± 0.40
	53-250	-17.3 ± 0.97	-17.9 ± 0.69	-18.8 ± 0.21	-16.9 ± 0.27	-17.7 ± 0.40	-17.8 ± 0.64	-18.5 ± 0.64	-18.1 ± 0.48	-16.6 ± 0.21	-17.3 ± 0.43	-17.7 ± 1.03	-16.8 ± 1.04
	<53	-17.2 ± 0.81	-17.9 ± 0.54	-18.5 ± 0.26	-17.4 ± 0.34	-17.4 ± 0.13	-17.5 ± 0.62	-18.9 ± 0.19	-18.7 ± 0.38	-16.5 ± 0.13	-16.6 ± 0.63	-18.4 ± 0.89	-17.5 ± 0.86
15-30													
	>2000	-16.7 ± 0.54	-16.9 ± 0.23	-9.5 ± 0.98	-13.5 ± 3.84	-15.0 ± 0.81	-14.0 ± 1.87	-14.1 ± 2.27	-14.5 ± 0.88	-15.3 ± 1.41	-17.0 ± 2.53	-16.8 ± 0.21	-13.1 ± 1.41
	250-2000	-16.3 ± 0.39	-17.6 ± 0.31	-16.9 ± 0.58	-16.1 ± 0.37	-16.2 ± 0.55	-16.8 ± 0.19	-17.3 ± 0.44	-16.6 ± 0.42	-16.2 ± 0.37	-18.0 ± 0.50	-17.9 ± 0.46	-16.4 ± 0.74
	53-250	-16.8 ± 0.34	-16.9 ± 0.41	-16.4 ± 0.24	-16.7 ± 0.59	-16.4 ± 0.53	-16.8 ± 0.53	-17.5 ± 0.10	-17.3 ± 0.47	-16.4 ± 0.25	-17.0 ± 0.57	-16.7 ± 0.70	-16.6 ± 0.32
	<53	-16.5 ± 0.33	-16.6 ± 0.53	-16.6 ± 0.15	-16.9 ± 0.36	-16.3 ± 0.36	-16.5 ± 0.42	-17.6 ± 0.16	-17.7 ± 1.07	-16.5 ± 0.39	-16.6 ± 0.57	-16.9 ± 0.55	-17.1 ± 0.45

Appendix 4. Temporal variation in the amount of shoot-derived ^{13}C incorporated into different aggregate size classes amended with wheat, pea and vetch shoot labeled residues (SL) at three soil depths (0-5, 5-15 and 15-30cm) under no-till field conditions (means ± SE).

Depth	Size	Wheat				Pea				Vetch			
		Days				Days				Days			
cm	µm	0	60	180	365	0	60	180	365	0	60	180	365
		mg cyinder^{-1}											
0-5													
	>2000	0	0.52 ± 0.08	0.32 ± 0.02	0.33 ± 0.13	0	0.35 ± 0.21	0.30 ± 0.15	0.33 ± 0.10	0	0.47 ± 0.14	0.35 ± 0.10	0.28 ± 0.21
	250-2000	0	0.04 ± 0.01	0.07 ± 0.02	0.07 ± 0.05	0	0.03 ± 0.02	0.07 ± 0.02	0.09 ± 0.06	0	0.02 ± 0.01	0.08 ± 0.05	0.09 ± 0.05
	53-250	0	0.06 ± 0.01	0.09 ± 0.04	0.15 ± 0.01	0	0.05 ± 0.02	0.09 ± 0.01	0.24 ± 0.10	0	0.05 ± 0.01	0.11 ± 0.04	0.31 ± 0.19
	<53	0	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0	0.02 ± 0.01	0.03 ± 0.01	0.07 ± 0.04	0	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.02
5-15													
	>2000	0	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	0	0.05 ± 0.02	0.02 ± 0.01	0.04 ± 0.03	0	0.07 ± 0.05	0.02 ± 0.02	0.05 ± 0.04
	250-2000	0	0.09 ± 0.02	0.02 ± 0.01	0.13 ± 0.05	0	0.09 ± 0.02	0.04 ± 0.02	0.12 ± 0.09	0	0.09 ± 0.052	0.02 ± 0.01	0.32 ± 0.03
	53-250	0	0.04 ± 0.01	0.02 ± 0.02	0.12 ± 0.06	0	0.03 ± 0.01	0.02 ± 0.02	0.23 ± 0.10	0	0.04 ± 0.03	0.01 ± 0.01	0.20 ± 0.02
	<53	0	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0	0.03 ± 0.02	0.01 ± 0.01	0.02 ± 0.02
15-30													
	>2000	0	0.04 ± 0.03	0.03 ± 0.01	0.04 ± 0.01	0	0.04 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0	0.05 ± 0.02	0.05 ± 0.03	0.04 ± 0.01
	250-2000	0	0.09 ± 0.08	0.05 ± 0.03	0.17 ± 0.04	0	0.04 ± 0.05	0.03 ± 0.01	0.28 ± 0.02	0	0.05 ± 0.05	0.06 ± 0.02	0.37 ± 0.01
	53-250	0	0.07 ± 0.06	0.03 ± 0.03	0.13 ± 0.09	0	0.03 ± 0.01	0.02 ± 0.03	0.15 ± 0.12	0	0.06 ± 0.04	0.02 ± 0.01	0.19 ± 0.04
	<53	0	0.05 ± 0.03	0.01 ± 0.01	0.06 ± 0.01	0	0.03 ± 0.02	0.02 ± 0.01	0.06 ± 0.01	0	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.01

Appendix 5. Temporal variation in the amount of root-derived ^{13}C incorporated into different aggregate size classes amended with wheat, pea and vetch root labeled residues (root+soil) at three soil depths (0-5, 5-15 and 15-30cm) under no-till field conditions (means ± SE).

Depth	Size	Wheat				Pea				Vetch			
		Days				Days				Days			
cm	µm	0	60	180	365	0	60	180	365	0	60	180	365
		mg cyinder^{-1}											
0-5													
	>2000	1.49 ± 0.30	2.01 ± 1.14	1.51 ± 0.02	1.63 ± 0.46	0.75 ± 0.10	1.62 ± 0.28	0.96 ± 0.05	0.98 ± 0.14	0.99 ± 0.20	2.44 ± 0.18	2.00 ± 0.45	1.47 ± 0.12
	250-2000	0.10 ± 0.04	0.14 ± 0.14	0.16 ± 0.02	0.37 ± 0.04	0.10 ± 0.01	0.15 ± 0.09	0.15 ± 0.05	0.20 ± 0.04	0.09 ± 0.02	0.13 ± 0.04	0.25 ± 0.06	0.34 ± 0.07
	53-250	0.10 ± 0.03	0.11 ± 0.03	0.13 ± 0.04	0.24 ± 0.05	0.10 ± 0.03	0.09 ± 0.02	0.09 ± 0.01	0.18 ± 0.04	0.10 ± 0.02	0.18 ± 0.08	0.19 ± 0.03	0.27 ± 0.03
	<53	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.04 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.08 ± 0.02
5-15													
	>2000	0.14 ± 0.07	0.21 ± 0.04	0.43 ± 0.11	0.22 ± 0.17	0.23 ± 0.14	0.28 ± 0.11	0.08 ± 0.11	0.24 ± 0.17	0.43 ± 0.07	0.65 ± 0.24	0.39 ± 0.21	0.31 ± 0.24
	250-2000	0.37 ± 0.18	0.24 ± 0.14	0.15 ± 0.08	0.51 ± 0.05	0.39 ±0.09	0.15 ± 0.12	0.09 ± 0.05	0.30 ± 0.10	0.62 ± 0.08	0.47 ± 0.03	0.33 ± 0.17	0.61 ± 0.08
	53-250	0.16 ± 0.12	0.09 ± 0.06	0.02 ± 0.02	0.19 ± 0.03	0.10 ± 0.02	0.08 ± 0.04	0.03 ± 0.04	0.08 ± 0.04	0.16 ± 0.02	0.12 ± 0.04	0.12 ± 0.10	0.23 ± 0.10
	<53	0.07 ± 0.02	0.04 ± 0.02	0.01 ± 0.01	0.07 ± 0.02	0.06 ± 0.01	0.04 ± 0.02	0.01 ± 0.01	0.02 ± 0.01	0.08 ± 0.01	0.08 ± 0.02	0.03 ± 0.03	0.06 ± 0.03
15-30													
	>2000	0.11 ± 0.02	0.12 ± 0.07	0.32 ± 0.02	0.08 ± 0.06	0.24 ± 0.09	0.20 ± 0.04	0.29 ± 0.15	0.15 ± 0.04	0.18 ± 0.07	0.18 ± 0.20	0.14 ± 0.01	0.11 ± 0.03
	250-2000	0.51 ± 0.12	0.21 ± 0.07	0.37 ± 0.13	0.54 ± 0.09	0.64 ± 0.12	0.45 ± 0.06	0.31 ± 0.10	0.49 ± 0.14	0.56 ±1.15	0.11 ± 0.13	0.14 ± 1.12	0.48 ± 1.18
	53-250	0.16 ± 0.05	0.13 ± 0.10	0.20 ± 0.04	0.20 ± 0.10	0.19 ± 0.06	0.15 ± 0.07	0.04 ± 0.01	0.08 ± 0.06	0.18 ± 0.04	0.12 ± 0.07	0.14 ± 0.08	0.19 ± 0.05
	<53	0.09 ± 0.01	0.09 ± 0.02	0.09 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.04 ± 0.01	0.04 ± 0.05	0.08 ± 0.01	0.08 ± 0.03	0.08 ± 0.02	0.05 ± 0.02