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

PRODUÇÃO DE BIOMASSA, COMPOSIÇÃO QUÍMICA E DECOMPOSIÇÃO DE RESÍDUOS CULTURAIS DA PARTE AÉREA E RAÍZES NO SOLO

TESE DE DOUTORADO

Marciel Redin

Santa Maria, RS, Brasil

2014

PRODUÇÃO DE BIOMASSA, COMPOSIÇÃO QUÍMICA E DECOMPOSIÇÃO DE RESÍDUOS CULTURAIS DA PARTE AÉREA E RAÍZES NO SOLO

Marciel Redin

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
2014

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

Redin, Marciel
Produção de biomassa, composição química e decomposição de resíduos culturais da parte aérea e raízes no solo / Marciel Redin.-2014.
114 f.; 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, 2014

1. Qualidade do resíduo 2. Mineralização 3. Carbono 4. Nitrogênio 5. Raízes I. José Giacomini, Sandro II. Aita, Celso III. Título.

 Endereço: Universidade Federal de Santa Maria, Centro de Ciências Rurais, Departamento de Solos, Av. Roraima, nº 1000, Cidade Universitária, Bairro Camobi, Santa Maria, RS, CEP 97105-900.
Fone/Fax (055) 3220 - 8108; End. Eletr: marcielredin@gmail.com

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

PRODUÇÃO DE BIOMASSA, COMPOSIÇÃO QUÍMICA E DECOMPOSIÇÃO DE RESÍDUOS CULTURAIS DA PARTE AÉREA E RAÍZES NO SOLO

elaborada por
Marciel Redin

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

COMISSÃO EXAMINADORA:

Sandro José Giacomini, Dr.
(Presidente/Orientador)

Celso Aita, Dr. (UFSM)

Gustavo Brunetto, Dr. (UFSM)

Claudia Pozzi Jantalia, Dr. (EMBRAPA)

Ricardo Bergamo Schenato, Dr. (UFSM)

Santa Maria, 27 de fevereiro de 2014.

DEDICATÓRIA

A minha família

A minha esposa
Luciana

AGRADECIMENTOS

Agradeço a Deus por guiar meus horizontes, inspirar forças, pelo que conquistei até agora e esperanças para superar novos desafios.

Ao povo brasileiro por fomentar a UFSM e concessão de bolsas de estudos através do CNPq, o que possibilitou a realização deste curso.

Ao professor Sandro José Giacomini, pela orientação, confiança em mim depositada, amizade e por incentivar-me a realizar Doutorado "*Sandwich"* no exterior, o que enriqueceu muito a minha formação profissional, pessoal e cultural.

A pesquisadora Drª. Sylvie Recous pelo profissionalismo, participação na elaboração das publicações, orientação e fundamental ajuda durante a realização do Doutorado "*Sandwich"* no INRA-Reims/França.

Ao professor Celso Aita, pela co-orientação, pelas sábias palavras, experiência, ensinamentos, idéias e sugestões durante o curso e também pela amizade.

Aos pesquisadores, funcionários e colegas de pós-graduação do "*Institut National de la Recherche Agronomique"* (INRA), unidade de Reims, França.

Aos bolsistas, ex-bolsistas entre outros do LABCEN – Grupo do professor Sandro: Pela amizade e auxílio nos experimentos de campo e de laboratório. Certamente, foi à ajuda de vocês que possibilitou a realização deste trabalho.

Aos bolsistas, ex-bolsistas, colegas entre outros do LABCEN – Grupo do professor Aita: Pela amizade, momentos de descontração, festas, futebol e churrascos.

Aos professores, funcionários, colegas de Mestrado, Doutorado e Pós-doutorado do Departamento de Solos e do PPGCS pela ajuda, amizade e pelo convívio diário.

As "minhas duas famílias" pelo incentivo, motivação, auxílio nos estudos e compreensão pelos longos períodos de ausência.

A minha esposa Luciana, sempre companheira em momentos bons e também de dificuldades no Brasil ou durante minha estadia no exterior. Obrigado pelo amor, palavras de motivação, incentivo e compreensão nos momentos de ausência.

Enfim, agradeço a todos que estiveram ao meu lado durante essa longa caminhada, me incentivando e dando forças, pois, se consegui chegar aqui, com certeza foi com a ajuda de todos vocês.

A todos, MUITO OBRIGADO!

RESUMO

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

PRODUÇÃO DE BIOMASSA, COMPOSIÇÃO QUÍMICA E DECOMPOSIÇÃO DE RESÍDUOS CULTURAIS DA PARTE AÉREA E RAÍZES NO SOLO

AUTOR: MARCIEL REDIN
ORIENTADOR: SANDRO JOSÉ GIACOMINI
Local e Data da Defesa: Santa Maria, 27 de fevereiro de 2014.

Os resíduos culturais (RC) da parte aérea (PA) e raízes (R) constituem a principal fonte de carbono (C) e nitrogênio (N) para os solos agrícolas. Comparado à PA das plantas poucos são os estudos sobre a produção de matéria seca (MS), acúmulo de C e N e decomposição das R. A decomposição de RC no solo é afetada em parte pela sua composição química, a qual pode ser afetada pela família e mistura de RC. Os objetivos do presente trabalho foram os seguintes: a) avaliar a produção de MS, composição química e acúmulo de C e N na MS da PA e de R de 27 espécies de culturas anuais de cinco famílias; b) relacionar a composição química das R de 20 espécies de culturas anuais com a mineralização do C no solo e; c) estudar as interações de aditividade ou não aditividade sobre a mineralização do C e do N de RC de diferentes composições químicas (folhas e talos) na superfície do solo. Foram realizados três estudos. No primeiro estudo foi quantificada a MS, acúmulo de C e N na MS, composição química da PA (folhas e talos) e R e nos outros dois, avaliou-se em condições de laboratório por 120 dias, a mineralização do C e/ou N dos RC no solo. Os dados medidos de mineralização do C foram ajustados com um modelo exponencial simples. Análises de componentes principais (ACPs) foram realizadas para explorar padrões qualitativos nos RC e de decomposição. A MS das R, concentradas na camada de 0-10 cm variou de 0,54 a 1,44 Mg ha^{-1} nas espécies de leguminosas (Fabaceae) e de 0,53 a 2,32 Mg ha^{-1} em não leguminosas (principalmente Poaceae), correspondendo a valores entre 63 a 97% da quantidade total de raízes da camada 0-20 cm. A relação R/PA média das leguminosas foi inferior àquela das espécies não leguminosas (0,14 vezes 0,20). As leguminosas acumularam nas R 392 kg C ha^{-1} e 15 kg N ha^{-1} e nas não leguminosas 642 kg C ha^{-1} e 16 kg N ha^{-1}. ACPs mostraram que a composição química da PA e R diferenciaram as famílias de plantas. A mineralização do C das R variou muito em termos de cinética e na quantidade total de C mineralizado (36% a 59%). A constante de mineralização (*k*) foi negativamente correlacionada com hemicelulose e positivamente com o teor de N nas R. R de Poaceae com alto conteúdo de hemicelulose, celulose e baixo N total apresentaram baixo valor de *k* e mineralização cumulativa de C. A PA das leguminosas apresentaram elevado N total, compostos solúveis e grandes diferenças entre a composição química de folhas e talos. As Poaceae foram caracterizadas por elevado conteúdo de hemicelulose e celulose, pequena diferença entre folhas e talos e lenta decomposição. A mistura de folhas e talos mostrou, principalmente, efeitos aditivos (efeito nulo) na mineralização do C e N no solo. Efeito antagonista na mineralização do N foi observado na maioria das misturas de leguminosas com elevado teor de N e grande heterogeneidade entre folhas e talos. Espécies de não leguminosas produzem maior quantidade de MS e acúmulo de C (1,8 vezes), maior acúmulo de N nas R (1,3 vezes) e relação R/PA (1,6 vezes) que leguminosas. A decomposição de R das espécies de culturas anuais é controlada pelo conteúdo de celulose e hemicelulose. As interações de não aditividade relacionadas à decomposição de misturas de resíduos de folhas e talos são controladas pelo grau de heterogeneidade e disponibilidade de N nas misturas.

Palavras-chave: Qualidade do resíduo. Mineralização. Carbono. Nitrogênio. Raízes.

ABSTRACT

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

BIOMASS PRODUCTION, CHEMICAL COMPOSITION AND DECOMPOSITION OF CROP RESIDUES FROM SHOOT AND ROOTS IN SOIL

AUTHOR: MARCIEL REDIN
ADVISER: SANDRO JOSÉ GIACOMINI
Date and Place of Defense: Santa Maria, February 27, 2014.

Crop residues (CR) from shoots (S) and roots (R) of plants are the main source of carbon C (C) and nitrogen (N) for the agricultural soils. Compared to the S of plants there are few studies on the production of dry matter (DM), C and N accumulation and decomposition of R. The decomposition of CR in soil is affected in part by its chemical composition, which can be affected by family and mixture of CR. The objectives of this study were as follows: a) evaluate the dry matter production (DM), chemical composition and accumulation of C and N in DM of S and R from 27 species of annual crops five families; b) relate the chemical composition of R from 20 species of annual crops with C mineralization in soil and c) study the interactions of additivity or non-additivity of the mineralization of C and N of CR of different chemical compositions (leaves and stems) on the surface of the soil. Three studies were realized: In the first study was quantified DM, accumulation of C and N in DM, chemical composition of S (leaves and stems) and R and the other two study was evaluated under laboratory conditions for 120 days, the mineralization of C and/or N of the CR in soil. The data measured of C mineralization were adjusted with a simple exponential model. Principal components analyses (PCAs) were performed to explore the qualitative address in CR and of decomposition. The DM of R, concentrated in the 0-10 cm layer, ranged from 0.54 to 1.44 Mg ha^{-1} in the legumes (Fabaceae) species and from 0.53 to 2.32 Mg ha^{-1} in the non-legumes (mainly Poaceae), corresponding the values between 63 to 97% of the total amount of roots in the 0-20 cm layer. The R/S ratio average was below legumes compared to non-legumes (0.14 times 0.20). The legumes acumulated in R 392 kg C ha^{-1} and 15 kg N ha^{-1} and the non-legumes 642 kg C ha^{-1} and 16 kg N ha^{-1}. PCAs showed that the chemical composition of the S and R differentiated plant families. Mineralization of C of R ranged widely in terms of kinetics and the total amount of C mineralized (36% to 59%). The mineralization constant (*k*) was negatively correlated with hemicellulose and positively with the N content in R. R of Poaceae with high content of hemicellulose, cellulose and low total N showed a low value of *k* and cumulative mineralization C. The S of the legumes showed high total nitrogen, soluble compounds and greater differences between the chemical composition of leaves and stems. The Poaceae were characterized by high content of hemicellulose and cellulose, little difference between leaves and stems and slow decomposition. The mixture of leaves and stems showed mainly additives effects (no effect) in the mineralization of C and N in the soil. Antagonistic effect on N mineralization was observed in most Fabaceae mixtures with high content of N and heterogeneity between leaves and stems. Species of non-legumes produce more DM and C accumulation (1.8 times), high accumulation of N in R (1.3 times) and ratio R/S (1.6 times) than legumes. The decomposition of R species of annual crops is controlled by the content of cellulose and hemicellulose. The interactions of non-additivity related to decomposition of mixed of residues of leaves and stems are controlled by the degree of heterogeneity and N availability in the mixtures.

Keywords: Quality residue. Mineralization. Carbon. Nitrogen. Roots.

LISTA DE FIGURAS

ARTIGO I

ARTIGO II

ARTIGO III

LISTA DE TABELAS

ARTIGO I

ARTIGO II

ARTIGO III

LISTA DE ANEXOS

LISTA DE APÊNDICES

SUMÁRIO

1 INTRODUÇÃO GERAL

Os resíduos de plantas sustentam o crescimento e atividade de microorganismos heterotróficos (HOOKER; STARK, 2008), promovem a ciclagem de nutrientes e, quando deixados na superfície do solo, protegem o mesmo dos processos erosivos. Ainda, os resíduos de plantas são a principal fonte de carbono (C) para os solos, através dos quais são mantidos os estoques de matéria orgânica no solo (MOS) que melhoram as suas propriedades químicas, físicas e biológicas (JARECKI, 2003; RASSE, 2005), e também contribuem para a retenção do CO_2 atmosférico no solo (LAL, 2004).

Nos sistemas agrícolas, muitas espécies de culturas anuais são usadas em sucessão e rotação de culturas com geração de diferentes quantidades e tipos de resíduos (folhas, talos e raízes) que são decompostos no solo (ABIVEN et al., 2005). Os resíduos de plantas exercem influência direta na dinâmica da mineralização do C e N no solo (JENSEN et al., 2005). Comparado à parte aérea, pouco se conhece sobre o acúmulo de matéria seca (MS), C e N e composição química das raízes e, portanto, mais informações são necessárias. A carência de informações disponíveis sobre raízes das plantas é decorrente da necessidade do uso de métodos trabalhosos e demorados empregados na coleta das raízes (AMOS; WALTERS, 2006). Com base nos valores de matéria seca da parte aérea e das raízes é possível calcular a relação raiz/parte aérea (R/PA) das plantas, a qual pode ser utilizada para estimar a produção de raízes a partir da MS aérea das plantas. Ainda, em muitos casos, a entrada de C de raízes no solo é calculada usando a relação R/PA (BOLINDER et al., 1999).

As raízes das culturas anuais podem representar uma grade proporção de material orgânico fresco que se decompõe no solo (ABIVEN et al., 2005) e são as principais responsáveis pela alocação de C em camadas inferiores do solo (BODDEY et al., 2010). Apesar da importância das raízes, apenas 2% dos estudos são relacionados à decomposição desse órgão das plantas no solo (ZHANG et al., 2008). Devido ao restrito e limitado número de espécies de culturas estudadas torna-se difícil estabelecer uma relação genérica entre as características químicas das raízes e a sua decomposição no solo para diversas espécies. A fração de C das raízes que vai permanecer no solo depende da quantidade de C mineralizado de seus tecidos durante a decomposição. Raízes que apresentam baixa decomposição podem resultar em alto sequestro de C no solo (RASSE et al., 2005; KONG; SIX, 2010). O conhecimento da dinâmica e a

quantidade de C mineralizado das raízes de diferentes espécies de plantas é importante para melhor estimar a contribuição das raízes para o C no solo. Além disso, o conhecimento do acúmulo de C e N na MS nas raízes permite melhor auxiliar na escolha de espécies que apresentem maior potencial para o aporte de C e N ao solo.

Na maioria dos estudos a mineralização do C e N de folhas e talos de espécies de culturas anuais é analisada para cada órgão separadamente (p.ex., ABIVEN et al., 2005). No entanto, em condições de campo as folhas e talos são decompostos juntos em uma mistura (HÄTTENSCHWILER et al., 2005). Resíduos de alta qualidade (p.ex., folhas) geram um ambiente de alta disponibilidade de N e podem estimular a mineralização do C e N no solo de resíduos de baixa qualidade (p.ex., talos) (CHAPMAN et al., 1988). Todavia, o efeitos da interação (aditivos ou não aditivos) na mineralização do C e N são contraditórios e dependentes de fatores relacionados principalmente à proporção da mistura (SALAMANCA et al., 1988), transferência de N entre os componentes da mistura (BERGLUND et al., 2013) ou composição química heterogênea das misturas (HARGUINDEGUY et al., 2008).

A composição química dos resíduos de culturas anuais é dependente das espécies ou famílias (ROUMET et al., 2006) e podem fornecer informações importantes em estudos relacionados à decomposição de resíduos no solo (TRINSOUTROT et al., 2000; JENSEN et al., 2005; SALL et al., 2007). Resíduos de plantas são compostos basicamente dos mesmos componentes, porém em diferentes proporções (HADAS et al., 2004). De fato, as células das plantas são compostas de uma fração solúvel relatada ao conteúdo citoplasmático e uma fração insolúvel composta pelas paredes celulares. O conteúdo de paredes celulares é dependente da espécie de planta e grau de maturidade (MACHINET et al., 2009; ABIVEN et al., 2011). Assim, a composição química intrínseca dos tecidos de diferentes famílias e espécies de plantas pode controlar a taxa de decomposição dos resíduos no solo.

Em síntese, o presente trabalho fundamenta-se na necessidade de aprofundar conhecimentos em alguns aspectos ainda carentes de resultados em espécies de plantas anuais em condições de clima subtropical: a) acúmulo de MS, C e N de raízes, b) caracterização química de resíduos de parte aérea e raiz, c) decomposição de raízes no solo e d) interações de aditividade ou não aditividade relacionadas à decomposição na superfície do solo entre a mistura de resíduos de diferentes composições químicas (folhas e talos). Assim, foram conduzidos experimentos em condições de campo e de laboratório que deram origem a presente tese, a qual foi organizada em três artigos.

1.1 Hipóteses

a) Espécies de não leguminosas apresentam maior acúmulo de MS, C e N que espécies de Fabaceae.

b) As famílias das espécies de culturas anuais determinam as características químicas dos resíduos e exerce impacto direto sobre a mineralização do C e N de resíduos da parte aérea e raízes no solo.

c) As interações de aditividade ou não aditividade em misturas de resíduos são dependentes da respectiva composição química de folhas e talos e da sua proporção na mistura que afetam diretamente a dinâmica do C e N no solo.

1.2 Objetivo geral

Avaliar o acúmulo de MS, C e N e a decomposição de raízes e parte aérea de espécies de culturas anuais de diferentes famílias utilizadas em sistemas agrícolas no Sul do Brasil.

1.3 Objetivos específicos

a) Avaliar o acúmulo de MS, C e N de raízes e determinar a relação raiz/parte aérea de 27 espécies de culturas anuais de cinco famílias em plantio direto nas condições de clima subtropical.

b) Caracterizar os resíduos da parte aérea e raízes de 25 espécies de culturas anuais de cinco famílias em C e N totais e solúveis em água, fracionamento químico (Van Soest) e polifenóis solúveis.

c) Relacionar a composição química de raízes de 20 espécies de culturas anuais de quatro famílias com a mineralização do C no solo, de modo a avaliar o potencial de contribuição das raízes para o C do solo.

d) Avaliar o efeito da mistura de resíduos de diferentes composições químicas (folha e talo) de 25 espécies de culturas anuais de cinco famílias na mineralização do C e N na superfície do solo.

2 ARTIGO I – MATÉRIA SECA, RELAÇÃO RAIZ/PARTE AÉREA, CARBONO E NITROGÊNIO DE ESPÉCIES DE CULTURAS ANUAIS EM PLANTIO DIRETO[1]

2.1 Resumo

O objetivo deste estudo foi quantificar o acúmulo de matéria seca (MS), carbono (C) e nitrogênio (N) de raízes e determinar a relação raiz/parte aérea (R/PA) de diversas espécies de culturas anuais de diferentes famílias em plantio direto nas condições de clima subtropical. A MS das raízes e parte aérea foi avaliada no florescimento de 11 espécies de leguminosas (Fabaceae) e 16 não leguminosas (principalmente Poaceae) utilizadas como culturas principais e plantas de cobertura de solo de estação primavera/verão e outono/inverno na região Sul do Brasil. Monólitos de solo foram coletados a 0-10 e 10-20 cm de profundidade. As raízes foram separadas do solo com o uso de água corrente sob peneira de 1 mm. Os resíduos da parte aérea e raízes foram secos em estufa a 65ºC, pesados, moídos e determinados o conteúdo de C e N totais. A MS total de raízes variou de 0,54 a 1,44 Mg ha^{-1} nas espécies leguminosas e de 0,53 a 2,32 Mg ha^{-1} nas não leguminosas. A MS ficou concentrada na camada de 0-10 cm e próxima à linha de semeadura. A R/PA de leguminosas foi inferior aquela das não leguminosas (0,14 vezes 0,20). Espécies de leguminosas acumularam na MS das raízes 392 kg C ha^{-1} e 15 kg N ha^{-1} e não leguminosas 642 kg C ha^{-1} e 16 kg N ha^{-1}. A família das espécies de culturas anuais determina a produção de MS de raízes. A maior parte da MS de raízes está localizada na camada superficial do solo (0-10 cm). Espécies de não leguminosas produzem maior quantidade de MS e acúmulo de C (1,8 vezes), maior acúmulo de N nas raízes (1,3 vezes) e maior relação R/PA (1,6 vezes) que leguminosas.

Palavras-chave: Biomassa; Relação raiz/parte aérea; Fabaceae; Poaceae

2.2 Introducão

[1] Artigo elaborado de acordo com as normas da revista Plant and Soil (Anexo A).

Os solos agrícolas são componente importante dos ecossistemas terrestres, que além de possibilitarem a produção de alimentos podem contribuir para a redução do efeito estufa através do sequestro de C. A quantidade de C sequestrada anualmente nos solos depende principalmente das condições edafoclimáticas, das práticas de manejo do solo e das culturas utilizadas. O efeito das culturas está ligado à qualidade química e a quantidade da biomassa adicionada ao solo, sendo esse último aspecto determinante da quantidade anual de C adicionada nos solos. O C fixado na biomassa das culturas é considerado a principal fonte deste elemento para os solos, o qual é adicionado ao solo através dos resíduos culturais da parte aérea (p.ex. folhas, talos, órgãos reprodutivos) e raízes (p.ex. biomassa e rizodepozições) das plantas (Bolinder et al. 2007). Em sistemas de manejo do solo em que não ocorre o revolvimento do solo (p.ex. plantio direto) as raízes das plantas são as principais responsáveis pelas entradas de matéria orgânica do solo (MOS) em profundidade (Zibilske et al. 2005). A determinação da adição de C ao solo via raízes comparada com a da parte aérea é complexa e de difícil execução (Angers et al. 1995), consequentemente, a biomassa radicular tem sido muitas vezes negligenciada em estudos sobre a entrada de C via resíduos culturais. Um melhor conhecimento da contribuição das raízes de diversas culturas anuais de diferentes famílias no aporte de C ao solo é necessário para melhor caracterizar e predizer a dinâmica do C no solo.

Muitas espécies de culturas principalmente das famílias das Fabaceae (leguminosas) e não leguminosas são usadas em sistemas de sucessão e rotação de culturas, e a quantidade e proporção das partes dessas plantas que retornam ao solo dependem do tipo de colheita e das práticas agrícolas (Abiven et al. 2005). A contribuição das raízes ao aporte de C no solo pode representar de 23 a 45% da MS da parte aérea (Prakash et al. 2002). Tal índice varia em função da família (Roumet et al. 2008) das espécies e cultivares (Xu e Juma 1992), condições de cultivo, estágio fenológico, condições edafoclimáticas durante o crescimento das plantas (Bolinder et al. 1997) e método de separação das raízes (Amato e Pardo 1994). Estimativas de estudos recentes mostraram considerável contribuição das raízes em acumular C no solo (Bolinder et al. 2007; Dos Santos et al. 2011). Outro aspecto importante está relacionado ao acúmulo de nutrientes nas raízes. No caso do N o conteúdo presente nas raízes de maneira geral pode representar de 10-15% do N total da planta (Kumar e Goh 2000), podendo em espécies leguminosas esse valor atingir até 40% (Rochester et al. 1998). A partir dos valores de MS da parte aérea e das raízes é possível estimar a R/PA

das plantas. Esse índice pode ser utilizado para estimar a produção de raízes a partir da avaliação da MS aérea das plantas. A entrada de C das raízes após a colheita é usualmente calculada usando a estimativa desse índice (Bolinder et al. 1999).

Atualmente, ainda são escassas as informações sobre as raízes de culturas principais (produção de grãos), e principalmente, de plantas de cobertura de solo em sistemas agrícolas. A pouca informação disponível sobre R/PA são provenientes de estudos conduzidos na América do Norte (Canadá e EUA) em condições de cultivo e solo característicos de clima temperado (Anderson 1988; Bolinder et al. 1997; Bolinder et al. 2002; Bolinder et al. 2007). A falta de informações em condições de clima subtropical, em sistema de plantio direto com plantas de cobertura de solo, obriga comumente utilizar tais resultados como ponto de referência para estudos relacionados a entradas de C e N no solo. A utilização de dados médios de literatura, principalmente de espécies de Poaceae como aqueles gerados no Canadá e EUA, por não representar as condições de estudo, podem levar a resultados contrastantes. Assim, o objetivo deste estudo foi quantificar o acúmulo de MS, C e N de raízes e determinar a relação R/PA de diversas espécies de culturas anuais de diferentes famílias em plantio direto nas condições de clima subtropical. A hipótese é que a família das diferentes culturas anuais determina a produção de MS e espécies de Poaceae apresentam maior acúmulo de MS, C e N que espécies de Fabaceae.

2.3 Material e métodos

Dois experimentos de campo foram conduzidos na área experimental do Departamento de Solos da Universidade Federal de Santa Maria, estado do Rio Grande do Sul, Região Sul do Brasil. O clima da região é subtropical úmido com precipitação média anual de 1686 mm, temperatura média anual de 19,3ºC e altitude média de 90 m. Os solos onde foram cultivadas as espécies de sequeiro e a cultura do arroz são classificados como Argissolo Vermelho Distrófico arênico e Planossolo Háplico Eutrófico arênico, respectivamente (Embrapa 2006). As características químicas dos solos no início do experimento encontram-se na Tabela 1.

No presente estudo foram avaliadas 27 espécies de culturas anuais, sendo 11 leguminosas (Fabaceae) e 16 não leguminosas (12 Poaceae) utilizadas como culturas

principais (14 espécies) e plantas de cobertura de solo em estações de primavera/verão (6 espécies) e outono/inverno (7 espécies) (Tabela 2). Com exceção da cultura do arroz que foi implantada com revolvimento do solo e manejada por inundação, as demais espécies foram conduzidas e amostradas em sistema de plantio direto consolidado a mais de dez anos em condições de sequeiro e sem irrigação. As espécies foram semeadas manualmente em parcelas de 20 m^2 (4x5m) com objetivo de obter maior uniformidade e/ou distribuição das plantas na linha de semeadura. Para as espécies semeadas em covas (feijão de porco, mamona e mucuna cinza) foram utilizadas quatro sementes e após a emergência das plântulas, foram deixadas somente duas plantas em cada cova. O controle de pragas e doenças até o momento da coleta das raízes e da parte aérea foi realizada de acordo com a recomendação técnica de cada cultura. A adubação mineral (N-P-K) foi realizada de com base na análise de solo e nas recomendações da Comissão de Química e Fertilidade do Solo - RS/SC. As plantas invasoras foram controladas manualmente através do arranque periódico das mesmas no estágio inicial de seu desenvolvimento. Dessa forma, excluiu-se a possibilidade de contaminação por raízes de outras plantas na área em estudo.

A coleta das raízes e da parte aérea, com três repetições para cada cultura dentro da parcela, foi efetuada no estágio reprodutivo correspondente à plena floração das plantas. Nas culturas principais destinadas e produção de grãos foi realizada uma segunda coleta da parte aérea no estágio de maturação fisiológica (colheita). Para a coleta das raízes, foi utilizado o método da escavação, o qual consistiu na abertura de uma trincheira no solo (área de 0,09 a 0,36m^2), perpendicular à linha de semeadura, com largura transversal igual ao espaçamento entrelinhas (17 a 90 cm), sendo a metade de cada lado da linha das plantas (Tabela 2). Para as culturas semeadas em covas, foi coletado o solo correspondente à área de três covas. O solo foi amostrado nas camadas de 0-10 e 10-20 cm de profundidade. Com o propósito de estudar a distribuição da MS de raízes das espécies de estação quente (primavera/verão) na camada de solo de 0-10 cm, a área de coleta das raízes foi dividida em duas posições: a primeira com 50% da área de coleta próxima a linha de semeadura e/ou cova (de acordo com o espaçamento entrelinhas) e a segunda com os 50% restante da área, porém distante da linha de semeadura e/ou cova.

Os monólitos de solo (solo + raízes) foram retirados manualmente com auxílio de pá de corte e enxada. Juntamente com a coleta das raízes, foi realizada para todas as espécies a coleta da parte aérea das plantas. Inicialmente a separação das raízes

presentes no solo foi realizada através da catação manual e, posteriormente, o solo foi lavado com água corrente sob peneira com malha de 1 mm. Após a coleta e separação das raízes do solo, a parte aérea de todas as espécies, e os resíduos de raízes, livres de nódulos radiculares para as espécies leguminosas, foram secos em estufa a 65ºC até peso constante. Depois de secas, as raízes foram separadas pelo diâmetro (Ø) com auxílio de régua milimetrada em grossas (Ø≥2 mm) e finas (1<Ø<2 mm). Uma subamostra dos resíduos secos da parte aérea e raízes foram finamente moídas (<1 mm) em moinho Willey. Uma subamostra contendo 1g do material moído foi incinerada em mufla a 550ºC por quatro horas para expressar os resultados livres de cinzas. O conteúdo de C e N totais dos resíduos de raízes e parte aérea foi determinado em autoanalisador CHNS (modelo FlashEA 1112, Thermo Finnigan, Milan, Itália).

A relação R/PA foi calculada dividindo a MS total de raízes na camada de 0-20 cm pela MS da parte aérea no florescimento e também na colheita. O acúmulo de C e N totais na MS foi calculado multiplicando a quantidade de MS pelo conteúdo de C e N totais nos tecidos da parte aérea e raízes. O acúmulo de C e N na MS das raízes e parte aérea das plantas de cobertura foi calculado no estágio de florescimento das espécies. Para o acúmulo de C e N na MS da parte aérea das culturas principais foi calculado com base nos resíduos de colheita. O acúmulo de C e N nas raízes foi calculado proporcional a MS de raízes grossas e finas ou somente nas raízes finas para as espécies que apresentaram somente esse tipo de raízes. A partição do C e N nas plantas foi calculada considerando a MS total de raízes e parte aérea no florescimento. Os resultados foram submetidos ao teste Least Significant Difference (LSD) para a comparação de médias (P<0.05). O desvio padrão das médias também foi calculado (DP).

2.4 Resultados e discussão

2.4.1 Matéria seca, distribuição e proporção das raízes no solo

A produção média de MS da parte aérea no florescimento foi de 6,97 Mg ha^{-1} para espécies de Fabaceae (leguminosas) e 7,6 Mg ha^{-1} em não leguminosas (principalmente Poaceae) (Tabela 3). A MS da parte aérea na colheita foi inferior à MS

obsevada no florescimento (média 14%). O total de MS das raízes (0-20 cm) de todas as espécies avaliadas variou de 0,53 Mg ha^{-1} na mamona a 2,32 Mg ha^{-1} na cultura do arroz. Comparando a produção média de raízes, observa-se que a produção de MS de raízes nas não leguminosas foi 1,8 vezes maior que leguminosas (1,40 vezes 0,84 Mg ha^{-1}). Roumet et al. (2008) também observaram que a produção de MS de raízes nas espécies de Poaceae foi 1,5 vezes superior, por exemplo, a de leguminosas. Esse resultado pode estar relacionado à própria natureza da família das espécies que determina a produção de MS (Roumet et al. 2008). Rosolem et al. (2002) em trabalho de casa de vegetação constataram após 40 dias de crescimento que a MS média de leguminosas (crotalária juncea e crotalária spectabilis) foi aproximadamente 7 vezes menor do que a observada nas Poaceae (sorgo e milheto). No presente estudo, entre as espécies leguminosas, destaca-se o guandu anão e ervilhaca com uma produção de MS de raízes de 1,44 Mg ha^{-1} e 1,33 Mg ha^{-1}, respectivamente. Entre as espécies não leguminosas destacam-se a aveia amarela (2,05 Mg ha^{-1}), aveia preta (1,85 Mg ha^{-1}), girassol (1,85 Mg ha^{-1}) e sorgo (1,84 Mg ha^{-1}). A produção de raízes em g $planta^{-1}$ variou de 0,38g na cultura do milheto a 24,48g no milho. Segundo Amos e Walters (2006), esse parâmetro pode ser importante em estudos onde a população de plantas é conhecida, pois a MS de raízes pode ser estimada pela multiplicação da população de plantas por hectare pela MS de raízes de cada planta.

A maior proporção de MS de raízes foi encontrada na camada de 0-10 cm de profundidade, para todas as espécies, com valores compreendidos entre 63% (tremoço azul) e 96% (milheto) do total da MS de raízes quantificada na camada de 0-20 cm (Tabela 3). Os resultados mostram que na média de todas as culturas avaliadas, 84% da MS de raízes produzida pelas plantas concentram-se na camada superficial do solo (0-10 cm). Provavelmente, a maior contribuição de C proveniente das raízes será nas camadas superficiais do solo. A maior incidência de raízes nos primeiros centímetros de profundidade pode estar relacionado ao sistema de plantio direto, que em razão desse sistema apresentar boas propriedades químicas em superfície, principalmente valores adequados de pH, Ca e P do solo. Além disso, o plantio direto proporciona melhorias nas propriedades físicas, com maior quantidade de poros e fissuras nas camadas superficiais que são vias preferenciais ao crescimento de raízes no solo (Filho et al. 2001). Possivelmente, o valor médio de MS de raízes obtido para a camada de 0-10 cm de profundidade esteja superestimado já que foi avaliado até 20 cm de profundidade. No entanto, outros trabalhos que avaliaram a MS de raízes até profundidades maiores (Filho

et al. 2004; Bordin et al. 2008; Giacomini et al. 2009) também quantificaram a maior proporção de MS de raízes nas camadas superficiais do solo, o que indica que no presente trabalho a medida de 0-20 cm quantificou a maior parte das raízes. Por exemplo, no trabalho de Giacomini et al. (2009), raízes de milho foram quantificadas até 120 cm de profundidade e os resultados mostraram que 86% da MS de raízes foi quantificada na camada de 0-20 cm.

Entre os cereais de inverno a maior parte da MS das raízes (89%) está localizada na camada superficial do solo (0-10 cm). Esses resultados são similares aos encontrados por Bolinder et al. (1997) que avaliaram a produção de MS de raízes de 0-30 cm de profundidade, através da coleta de núcleos de solo, de diferentes espécies e cultivares de cereais de inverno no Canadá implantadas com revolvimento do solo. Esses autores constaram que aproximadamente 70% da MS de raízes encontram-se na camada de 0-15 cm do solo. No entanto, o trabalho de Pietola e Alakukku (2005) mostrou que a proporção relativa de MS total de raízes para 0-60 cm profundidade em cevada e trigo coletada na camada de 0-20 cm foi de 59 e 80%, respectivamente. Hansson e Andrén (1987) avaliaram raízes de cevada até 100 cm de profundidade e quantificaram aproximadamente 75% da MS de raízes na camada de 0-20 cm. No presente estudo, a diferença na produção de MS de raízes de cereais de inverno na camada superficial do solo, comparado aos resultados de outros estudos, além das características do plantio direto, pode estar relacionada com diferenças no clima, condições de solo e cultivares (Xu e Juma 1992; Pietola e Alakukku 2005).

A maior quantidade de MS de raízes para espécies de primavera/verão na camada de 0-10 cm foi mensurada na região próxima à linha de semeadura/covas (média 90%) (Figura 1). A maior concentração de raízes próxima à linha de semeadura/covas foi observada em sete espécies com destaque para a cultura da crotalária spectabilis e do girassol, com 98% e 97%, respectivamente. Skinner et al. (1998) constataram que 63% da MS total de raízes de milho estavam localizadas na região próxima da linha de semeadura. Bolinder et al. (1997), com cereais de inverno na maturidade fisiológica, encontraram 65% desta MS próxima à linha de semeadura e 35% nas entrelinhas. Por exemplo, 97% das raízes do girassol estiveram concentradas em 50% da área de abrangência da planta (próximo à linha de semeadura), pode-se estimar que a quantidade de raízes nessa área è de 1673 kg ha^{-1} e de apenas 52 kg ha^{-1} no restante da área (entrelinhas de semeadura). Essa variação espacial das raízes na camada superficial do solo deve provocar também uma variação espacial nos fluxos de

mineralização/imobilização dos nutrientes durante a decomposição das raízes no solo além de afetar a distribuição do C das raízes no interior do solo.

A proporção de raízes grossas e finas indica que na média de todas as espécies avaliadas, 61% da MS de raízes produzida são de raízes finas e apenas 39% de raízes grossas (Tabela 3). Não foram encontradas raízes grossas (Ø≥2 mm) para ervilha, ervilhaca, arroz, azevém as espécies de aveia e os cereais de inverno. As espécies de leguminosas, em média, apresentam maior quantidade de raízes grossas (59%) em relação às não leguminosas que apresentam maior proporção de raízes finas (74%). A maior proporção de raízes finas encontradas com as espécies não leguminosas, especialmente as Poaceae, deve-se ao fato destas culturas apresentarem um sistema radicular fasciculado, onde há o predomínio desse tipo de raiz (Roumet et al. 2008). Segundo Rasse et al. (2005) as raízes podem crescer no interior dos agregados, promovendo a proteção e estabilização do C no solo. Nesse sentido, espécies que apresentam maior proporção de raízes finas, podem contribuir mais para o C orgânico do solo devido a maior facilidade de penetrar e crescer no interior de agregados do solo. Whitely e Dexter (1984) observaram que em solos compactados as plantas com numerosas raízes finas (trigo) o crescimento radicular foi menos afetado em comparação às plantas com raízes mais grossas (girassol). Ainda, a característica física das raízes pode determinar a proporção de C e N no seu tecido, pode assumir grande influência nos processos de mineralização e imobilização de N mineral durante a sua decomposição no solo.

2.4.2 Relação R/PA

A relação R/PA na colheita foi superior (17%) ao observado na floração das culturas anuais principais, provavelmente devido a menor produção de MS da parte aérea na colheita (Tabela 3). No florescimento a relação R/PA foi em média de 0,18, com variação de 0,07 (feijão de porco) a 0,37 (ervilhaca). Esse índice médio foi de 0,13 para as espécies de leguminosas e de 0,18 para não leguminosas, o que indica que estas últimas compostas por um maior número de espécies de Poaceae promovem maior aporte de C ao solo através das raízes. Para os cereais de inverno, incluindo as espécies de aveia (preta, branca e amarela) o índice foi de 0,25. Esse resultado diverge daqueles apresentados por Bolinder et al. (2007) onde para essas espécies os autores encontram

um índice de 0,14. Entre as espécies de Poaceae o sorgo e milho apresentaram as maiores relações, 0,23 e 0,18, respectivamente. Para o milho, o valor da relação R/PA foi de 0,18, próximo ao encontrado no trabalho de revisão de Amos e Walters (2006) e igual à revisão de Bolinder et al. (2007) com relações de 0,16 (milho produção de grãos) e 0,18 (milho silagem). Entre as espécies leguminosas a ervilhaca apresentou a maior relação R/PA (0,37) o que evidencia sua alta produção de MS de raízes (Tabela 3). Esse resultado corrobora com o observado por Kuo et al. (1997) que encontraram R/PA de 0,33 para profundidade de 0-20 cm. No entanto, no trabalho de Puget e Drinkwater (2001) a relação foi de 0,26, e, portanto, inferior que o presente estudo. Tais divergências nos resultados podem ser explicadas pela menor produção de MS de raízes ou maior produção de MS da parte aérea nas condições de clima temperado ou pela maior profundidade de coleta das raízes.

De acordo com Bolinder et al. (2007), esse índice é importante, pois é usualmente utilizado para calcular a entrada de C da MS de raízes após a colheita a partir da avaliação da MS da parte aérea das plantas. No entanto, devido à elevada varição dos resultados disponíveis sobre MS, distribuição de raízes no solo e R/PA existe uma grande incerteza na adição de MS e nas estimativas de entrada de C e N, pelas raízes das plantas. Esse índice pode ser variável mesmo dentro das mesmas espécies e cultivares, pois é dependente de vários fatores. Segundo Pietola e Alakukku (2005) as diferenças na relação R/PA e outros parâmetros da parte aérea e das raízes, podem ser parcialmente explicados pelos diferentes métodos de amostragem e mensuração da MS de raízes. No entanto, Bolinder et al. (1997) citam um conjunto de fatores que podem influenciar a relação R/PA e produção de MS de raízes nos cereais de inverno. Segundo esses autores, tais diferenças podem ser explicadas pelas diferenças metodológicas (posição e profundidade de amostragem, métodos de separação das raízes), outras características específicas do local (regime de umidade do solo, temperatura, disponibilidade de N, textura do solo) e diferenças fisiológicas entre cultivares. No trabalho de Bolinder et al. (1997) a diferença na MS de raízes de três cultivares de cevada foi de aproximadamente 25%. Anderson (1988) estudou o efeito do revolvimento do solo e da adubação de N no crescimento e relação R/PA do milho. Os resultados mostraram que não foram observadas diferenças para o revolvimento do solo e a adubação com N decresceu significativamente a relação R/PA devido à parte aérea responder a adubação nitrogenada; no entanto, as raízes não foram influenciadas. Ainda, segundo Oliveira et al. (2000), tipicamente 20 a 40% do peso original das raízes podem

ser perdido durante a sua lavagem. Essa perda de peso pode influenciar a MS de raízes, e consequentemente, na relação R/PA.

2.4.3 Acúmulo de C e N na MS da parte aérea e raízes

O conteúdo de C na MS da parte aérea e raízes das plantas apresentou pouca variação em comparação ao N que apresentou maior variação (Tabela 4). De maneira geral, o conteúdo de N nos tecidos da parte aérea foi maior que nas raízes (30%). As raízes de leguminosas apresentaram maior conteúdo de N em comparação às espécies não leguminosas (54%). Essa variação no conteúdo de N nos tecidos explica a larga variação nas relações C:N encontradas na parte aérea e raízes (Tabela 4). Entre as espécies de não leguminosas o arroz (30,2 kg ha^{-1}), triticale (25,3 kg ha^{-1}) e sorgo (22,1 kg ha^{-1}) foram às culturas que apresentaram uma maior reciclagem de N. Conforme o modelo conceitual proposto por Kleber et al. (2007), o maior conteúdo de N nos tecidos de raízes de leguminosas, principalmente associada a sua forma orgânica, pode contribuir de forma mais eficiente para a MOS. Ainda, segundo Puget e Drinkwater (2001) o fato da natural renovação das raízes (turnover) e rizodeposições estas promovem uma contínua contribuição de C no solo.

O acúmulo de C na MS das raízes variou de 247 kg ha^{-1} no feijão de porco a 1027 kg ha^{-1} no arroz e N variou de 4,6 kg ha^{-1} na mamona a 50 kg ha^{-1} na ervilhaca. A quantidade acumulada de C e N nos resíduos da parte aérea foi 5,9 e 7,3 vezes superior a aquela de raízes, respectivamente. Estes resultados indicam claramente que o acúmulo de C e N é dependente da quantidade de MS produzida para cada espécie. A quantidade de N acumulado pela parte aérea das espécies leguminosas foi maior para o feijão de porco (325 kg ha^{-1}). Entre as espécies de leguminosas a ervilhaca acumulou com a parte aérea 141 kg ha^{-1} de N e foi à espécie que mais acumulou N nos tecidos de raízes (50 kg ha^{-1}). Esse resultado está relacionado à alta produção de MS das raízes (Tabela 3) e da eficiente fixação simbiótica de N nessa leguminosa. Resultado semelhante foi obtido por Ozpinar e Baytekin (2006) que observaram acúmulo total de N nas raízes de ervilhaca de 95 kg ha^{-1}. O maior acúmulo de N (55%) no trabalho desses autores provavelmente está relacionado ao tipo de preparo do solo, método, profundidade de coleta das raízes (40 cm) e maior produção de MS de raízes (2619 kg ha^{-1}). Esses

resultados evidenciam o potencial da ervilhaca em adicionar N para o solo através da parte aérea e, também pelas raízes. Nesse sentido, o uso de ervilhaca em esquemas de sucessão e rotação de culturas com cereais de inverno pode levar a redução do uso de fertilizante nitrogenado além de levar a maior produção de MS e grãos nas culturas principais em sucessão (Jones e Sing 2000; Yau et al. 2003).

A parte aérea do milheto (10334 kg ha^{-1}) e guandu anão (6288 kg ha^{-1}) acumularam as maiores quantidades de C entre não leguminosas e leguminosas, respectivamente. A adição de C para o solo via raízes foi maior (64%) para as espécies não leguminosas em comparação às leguminosas. As raízes de arroz acumularam a maior quantidade de C no solo (1027 kg ha^{-1}). Entre as espécies de sequeiro maiores quantidades adicionadas de C foram observadas com girassol (864 kg ha^{-1}), sorgo (856 kg ha^{-1}), aveia preta (855 kg ha^{-1}) e aveia amarela (812 kg ha^{-1}). Entre as leguminosas, o guandu anão (670 kg ha^{-1}) e ervilhaca (624 kg ha^{-1}). Esses resultados evidenciam a importância das Poaceae, como a aveia amarela, sorgo e aveia preta na incorporação de C ao solo através das raízes. Nesse aspecto também se destaca o girassol, que devido à elevada produção de MS de raízes com alta C:N (90), pode promover o aumento dos estoques de MOS. Entre as espécies de leguminosas, o guandu anão e a ervilhaca reforçam também a importância da inclusão dessas espécies em esquemas de sucessão e rotação de culturas, a fim de aumentar o aporte de C ao solo. Ainda, a elevada produção de MS da parte aérea do milheto e guandu anão entre as espécies de não leguminosas e leguminosas, respectivamente, pode ser uma boa alternativa para aportar C ao solo além de cobertura e proteção do solo contra a erosão dos solos.

Da mesma forma que a relação R/PA, através da relação entre o conteúdo de C e N acumulado nas raízes e parte aérea, pode-se estimar a quantidade que seria adicionada de C e N no solo pelas raízes a partir do conhecimento da MS e conteúdo de C e N da parte aérea das plantas. Em média para todas as espécies essa relação foi de 0,29 para C e de 0,36 para o N (Tabela 4). Esses resultados estão de acordo com os relatados por Prakash et al. (2002) e Rochester et al. (1998) para C e N, respectivamente. Em função das espécies leguminosas apresentarem menor produção de MS de raízes, a relação entre o N das raízes e o N da parte aérea foi 1,4 vezes inferior em comparação às espécies não leguminosas que apenas reciclam e acumulam o N na parte aérea e raízes. Para o C as variações de adição entre as raízes e parte aérea foram dependentes da MS produzida, pois o conteúdo de C na MS de raízes e parte aérea é pouco variável.

Embora a maior quantidade de C e N seja adicionada pela parte aérea das plantas o N, e principalmente, o C presente nas raízes pode contribuir mais para a formação e o estoque de MOS em função das características químicas dos resíduos, proteção física e química no interior de agregados. Em trabalho de revisão, Rasse et al. (2005) concluíram que os resultados de experimentos *in situ* indicam que a contribuição da raiz para C orgânico do solo é, em média, 2,4 vezes maior do que a parte aérea. No entanto, na análise realizada por Rasse et al. (2005), e no estudo de Bolinder et al. (2002), foi assumido que a entrada de C pelas raízes seria equivalente à da MS de raízes mensurada, sem considerar as rizodeposições. Bolinder et al. (2007), ao estimar a produtividade primária e entrada anual de C para o solo no Canadá assumiram que as rizodeposições seriam correspondentes a 65% da MS de raízes. Kätterer et al. (2011), que assumiram o índice proposto por Bolinder et al. (2007) verificaram em experimento de longa duração, que o coeficiente de C derivado das raízes foi 2,3 vezes maior que os resíduos da parte aérea.

2.5 Conclusões

A família das espécies de culturas anuais determina a produção de MS de raízes. A maior parte da MS de raízes está localizada na camada superficial do solo (0-10 cm). Espécies de não leguminosas produzem maior quantidade de MS e acúmulo de C (1,8 vezes), maior acúmulo de N nas raízes (1,3 vezes) e maior relação R/PA (1,6 vezes) que leguminosas. No entanto, leguminosas acumulam maior quantidade N por unidade de C da MS de raízes.

Agradecimentos

Este trabalho foi suportado pelo governo brasileiro através da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

2.6 Referências

Abiven S, Recous S, Reyes V, Oliver R (2005) Mineralization of C and N from root, stem and leaf residues in soil and role of their biochemical quality. Biol Fertil Soils 45:119-128

Amos B, Walters, DT (2006) Maize Root Biomass and Net Rhizodeposited Carbon: An Analysis of the Literature. Soil Sci Soc Am J 70:1489-1503

Amato M, Pardo A (1994) Root length and biomass losses during sample preparation with different screen mesh sizes. Plant Soil 161:299-303

Anderson EL (1988) Tillage and N fertilization effects on maize root growth and root: shoot ratio. Plant Soil 108: 245-251

Angers DA, Voroney RP, Côté D (1995) Dynamics of soil organic matter and corn residues affected by tillage practices. Soil Sci Soc Am J 59:1311-1315

Bolinder MA, Angers DA, Dub JP (1997) Estimating shoot to root ratios and annual carbon inputs in soils for cereal crops. Agr Ecosyst Environ 63:61-66

Bolinder MA, Angers DA, Belanger G, Michaud R, Laverdiere MR (2002) Root biomass and shoot to root ratios of perennial forage crops in eastern Canada. Can J Plant Sci 82:731-737

Bolinder MA, Janzen HH, Gregorich EG, Angers DA, VandenBygaart AJ (2007) Na approach for estimating net primary productivity and annual carbon inputs to soil for common agricultural crops in Canada. Agr Ecosyst Environ 118:29-42

Bordin I, Neves CSVJ, Medina CC, Dos Santos JCF, Torres E, Urquiaga S (2008) Matéria seca, carbono e nitrogênio de raízes de soja e milho em plantio direto e convencional. Pesq Agropec Bras 43:1785-1792

Dos Santos NZ, Dieckow J, Bayer C, Molin R, Favaretto N, Pauletti V, Piva JT (2011) Forages, cover crops and related shoot and root additions in no-till rotations to C sequestration in a subtropical Ferralsol. Soil Till Res 111:208-218

Empresa Brasileira de Pesquisa Agropecuária. Centro Nacional de Pesquisa de Solos (Rio de Janeiro, RJ). Sistema Brasileiro de Classificação de Solos. 2. ed. Brasília: Embrapa Produção de Informação, 2006. 306p

Filho JT, Barbosa GMC, Guimarães MF, Fonseca ICB (2001) Resistência à penetração e desenvolvimento do sistema radicular do milho (*Zea mays*) sob diferentes sistemas de manejo em Latossolo Roxo. R Bras Ci Solo 25:725-730

Filho SPV, Brigitte JF, Piccolo MC, Fante Jr L, Neto MS, Cerri CC (2004) Root systems and soil microbial biomass under no-tillage system. Sci Agric 61:529-537

Giacomini SJ, Aita C, Jantalia CP, Urquiaga S (2009) Aproveitamento pelo milho do nitrogênio amoniacal de dejetos líquidos de suínos em plantio direto e preparo reduzido do solo. Pesq Agropec Bras 44:761-768

Hansson AC, Andrén O (1987) Root dynamics in barley, lucerne and meadow fescue investigated with a mini-rhizotron technique. Plant Soil 103:33-38

Jones MJ, Sing M (2000) Long-term yield patterns in barley-based cropping systems in Northern Syria. 2. The role of feed legumes. J Agric Sci 135:237-249

Kätterer T, Bolinder MA, Andrén O, Kirchmann H, Menichetti L (2011) Roots contribute more to refractory soil organic matter than above-ground crop residues, as revealed by a long-term field experiment. Agric Ecosyst Environ 141:184-192

Kleber M, Sollins P, Sutton R (2007) A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces. Biogeochemistry 85:9-24

Kumar K, Goh KM (2000) Biological nitrogen fixation, accumulation of soil nitrogen and nitrogen balance for white clover (*Trifolium repens* L.) and field pea (*Pisum sativum* L.) grown for seed. Field Crop Res 68:49-59

Kuo S, Sainju UM, Jellum EJ (1997) Winter cover cropping influence on nitrogen in soil. Soil Sci Soc Am J 61:1392-1399

Oliveira MRG, Van Noordwijk M, Gaze SR, Brouwer G, Bona S, Mosca G, Hairiah K (2000) Auger sampling, ingrowth cores and pinboard methods. In: Smit AL, Bengough AG, Engels C, Van Noordwijk M, Pellerin S, Van de Geijn SC (Eds.). Root Methods: A Handbook. Springer-Verlag, Berlin, pp. 175-210

Ozpinar S, Baytekin H (2006) Effects of tillage on biomass, roots, N-accumulation of vetch (*Vicia sativa* L.) on a clay loam soil in semi-arid conditions. Field Crop Res 96:235-242

Pietola L, Alakukku L (2005) Root growth dynamics and biomass input by Nordic annual field crops. Agr Ecosyst Environ 108:135-144

Prakash V, Kundu S, Ghosh BN, Singh RD, Gupta HS (2002) Annual carbon input to soil through rainfed soybean (*Glycine max*) - wheat (*Triticum aestivum*) cropping sequence in mid-hills of North-West Himalaya. Indian J Agr Sci 72:14-17

Puget P, Drinkwater LE (2001) Short-term dynamics of root- and shoot-derived carbon from a leguminous green manure. Soil Sci Soc Am J 65:771-779

Rasse DP, Rumpel C, Dignac MF (2005) Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant Soil 269: 341-356

Rochester I, Peoples MB, Constable GA, Gault R (1998) Faba beans and other legumes add nitrogen to irrigated cotton cropping systems. Australian J Exp Agric 38:253-260

Rosolem CA, Foloni JSS, Tiritan CS (2002) Root growth and nutrient accumulation in cover crops as affected by soil compaction. Soil Till Res 65:109-115

Roumet C, Lafont F, Sari M, Warembourg F, Garnier E (2008) Root traits and taxonomic affiliation of nine herbaceous species grown in glasshouse conditions. Plant Soil 312:69-83

Skinner RH, Hanson JD, Benjamin JG (1998) Root distribution following spatial separation of water and nitrogen supply in furrow irrigated corn. Plant Soil 199:187-194

Whitely GM, Dexter AR (1984) The behaviour of root encountering cracks in soil. I. Experimental methods and results. Plant Soil 77:141-149

Xu JG, Juma NG (1992) Above and below ground transformation of photosynthetically fixed carbon by two barley (*Hordeum vulgare* L.) cultivars in a Typic Cryoboroll. Soil Biol Biochem 25:1263-1272

Zibilske LM, Materon LA (2005) Biochemical properties of decomposition cotton and corn stem and root residues. Soil Sci Soc Am J 69:378-386

Yau SK, Bounejmate M, Ryan J, Baalbaki R, Nassar A, Maacaroun R (2003) Barley legumes for semi-arid areas of Lebanon. Eur J Agron 19:599-610

Tabela 1: Características químicas dos solos nas áreas experimentais onde as espécies de culturas anuais foram cultivadas.

Solo	Profundidade (cm)	Densidade (g cm^{-3})	pH[a] (Água)	V (%)	MOS	Argila	P	K	H+Al	Ca	Mg
					--- g kg^{-1} ---		--- mg kg^{-1} ---		--- $mmol_c$ dm^{-3} ---		
Argissolo	0-10	1,54	5,4	74,5	16,1	170,4	13,5	156,4	2,5	2,9	3,7
	10-20	1,55	5,3	70,8	12,4	160,6	6,9	40,3	2,5	2,4	3,3
Planossolo	0-10	1,32	4,3	46,3	22,2	290,3	8,4	100,4	9,7	6,2	1,8
	10-20	1,36	4,5	43,5	14,9	300,6	11,8	40,2	12,3	6,9	2,2

[a] pH: Potencial hidrogeniônico; V: Saturação por bases; MOS: Matéria orgânica do solo; P: Fósforo; K: Potássio; H+Al: Acidez potencial; Ca: Cálcio; Mg: Magnésio.

Tabela 2: Descrição das espécies de culturas anuais, adubação mineral, espaçamento entrelinhas (EL), área (AC) e plantas coletadas (PC) por repetição.

Espécies	Nome científico	Família	Uso agrícola	EL (cm)	AC (m^2)	PC	N-P-K[a]
Leguminosas							
Crotalária juncea	*Sunn hemp*	Fabaceae	Planta cobertura	40	0,20	12	0-0-0
Crotalária spectabilis	*Crotalaria spectabilis*	Fabaceae	Planta cobertura	30	0,15	15	0-0-0
Feijão	*Phaseolus vulgaris*	Fabaceae	Cultura principal	45	0,17	3	45-13-14
Feijão de porco	*Canavalia ensiformis*	Fabaceae	Planta cobertura	45	0,14	2	0-0-0
Guandu anão	*Cajanus cajan*	Fabaceae	Planta cobertura	40	0,20	7	0-0-0
Mucuna cinza	*Stizolobium niveum*	Fabaceae	Planta cobertura	40	0,15	2	0-0-0
Soja	*Glycine max*	Fabaceae	Cultura principal	45	0,19	5	0-13-41
Tremoço azul	*Lupinus angustifolius*	Fabaceae	Planta cobertura	17	0,09	5	0-10-25
Tremoço nativo	*Lupinus albescens*	Fabaceae	Planta cobertura	17	0,09	10	40-10-25
Ervilha	*Pisum arvensis*	Fabaceae	Planta cobertura	17	0,09	11	0-10-30
Ervilhaca	*Vicia sativa*	Fabaceae	Planta cobertura	20	0,10	25	0-10-30
Não leguminosas							
Girassol	*Helianthus annuus*	Asteraceae	Cultura principal	60	0,24	2	50-13-41
Mamona	*Ricinnus comunis*	Euphorbiaceae	Cultura principal	90	0,36	2	50-10-17
Milho	*Zea mays*	Poaceae	Cultura principal	90	0,36	2	105-25-35
Sorgo	*Sorghum bicolor*	Poaceae	Cultura principal	45	0,23	7	95-20-45
Milheto	*Pennisetum glaucum*	Poaceae	Planta cobertura	20	0,10	37	90-0-0
Canola	*Brassica napus oleifera*	Brassicaceae	Cultura principal	17	0,09	15	80-12-35
Nabo forrageiro	*Raphanus sativus oleiferus*	Brassicaceae	Planta cobertura	17	0,25	23	0-10-35
Arroz	*Oryza sativa*	Poaceae	Cultura principal	17	0,15	54	120-11-85
Aveia amarela	*Avena bysantina*	Poaceae	Cultura principal	17	0,09	14	87-8-30
Aveia branca	*Avena sativa*	Poaceae	Cultura principal	17	0,09	13	87-8-30
Aveia preta	*Avena strigosa*	Poaceae	Planta cobertura	17	0,09	18	87-12-35
Azevém	*Lolium multiflorum*	Poaceae	Planta cobertura	17	0,17	21	87-12-35
Centeio	*Secale cereale*	Poaceae	Cultura principal	17	0,17	18	87-12-35
Cevada	*Hordeum vulgare*	Poaceae	Cultura principal	17	0,09	19	87-12-35
Trigo	*Triticum aestivum*	Poaceae	Cultura principal	17	0,09	27	87-12-35
Triticale	*Triticosecale rimpaui*	Poaceae	Cultura principal	17	0,09	13	87-12-35

[a] N-P-K é o total da adubação em kg ha^{-1} de N (sulfato de amônio e uréia), fósforo (superfosfato triplo) e potássio (cloreto de potássio). 0-0-0: Fertilidade natural do solo (Tabela 1).

Tabela 3: Matéria seca da parte aérea, raízes e relação R/PA das espécies de culturas anuais.

(continua)

Espécies	Parte aérea (Mg ha^{-1})		Raízes					R/PA[a]	
	Florescimento	Colheita	0-10 (Mg ha^{-1})	0-20 (Mg ha^{-1})	0-10 (%)	Fina (%)[b]	Planta (g)	Florescimento	Colheita
Leguminosas									
Crotalária juncea	8,90		0,79	0,88	90	26	1,46	0,10 ± 0,02	
Crotalária spectabilis	7,21		0,51	0,59	87	4	0,59	0,08 ± 0,02	
Feijão	4,73	3,50	0,65	0,72	90	41	4,07	0,15 ± 0,03	0,21 ± 0,03
Feijão de porco	7,99		0,43	0,54	79	41	3,63	0,07 ± 0,02	
Guandu anão	12,22		1,24	1,44	86	9	4,12	0,12 ± 0,02	
Mucuna cinza	5,74		0,50	0,67	73	41	4,50	0,12 ± 0,02	
Soja	9,70	7,37	0,74	0,81	91	45	3,03	0,08 ± 0,03	0,11 ± 0,03
Tremoço azul	5,54		0,54	0,85	63	32	1,45	0,16 ± 0,04	
Tremoço nativo	5,51		0,50	0,76	64	12	0,69	0,14 ± 0,02	
Ervilha	5,43		0,45	0,66	68	100	0,51	0,12 ± 0,02	
Ervilhaca	3,67		1,09	1,33	82	100	0,54	0,37 ± 0,06	
Não leguminosas									
Girassol	12,05	9,19	1,73	1,85	93	31	22,15	0,15 ± 0,02	0,20 ± 0,03
Mamona	3,35	3,11	0,38	0,53	72	37	19,04	0,16 ± 0,04	0,18 ± 0,05
Milho	7,77	6,55	1,13	1,36	83	52	24,48	0,18 ± 0,04	0,21 ± 0,03
Sorgo	8,04	7,64	1,60	1,84	87	42	5,93	0,23 ± 0,03	0,24 ± 0,03
Milheto	22,48		1,55	1,60	97	73	0,38	0,07 ± 0,01	
Canola	2,76	2,38	0,66	0,84	77	46	0,49	0,31 ± 0,07	0,36 ± 0,09
Nabo forrageiro	6,81		0,79	0,86	92	11	0,95	0,13 ± 0,02	
Arroz	11,71	9,93	2,05	2,32	88	100	0,66	0,20 ± 0,03	0,23 ± 0,04
Aveia amarela	6,33	5,55	1,71	1,82	93	100	1,10	0,29 ± 0,05	0,33 ± 0,06
Aveia branca	4,75	4,23	1,25	1,36	91	100	0,89	0,29 ± 0,04	0,32 ± 0,04
Aveia preta	7,98		1,66	1,85	89	100	0,86	0,23 ± 0,04	
Azevém	5,48		0,99	1,15	86	100	0,96	0,21 ± 0,02	
Centeio	5,75	5,06	1,29	1,37	94	100	1,29	0,23 ± 0,04	0,27 ± 0,05
Cevada	5,71	4,79	0,92	1,07	92	100	0,46	0,18 ± 0,02	0,21 ± 0,03

Tabela 3: Matéria seca da parte aérea, raízes e relação R/PA das espécies de culturas anuais.

(conclusão)

Espécies	Parte aérea (Mg ha^{-1})		Raízes					R/PA[a]	
	Florescimento	Colheita	0-10 (Mg ha^{-1})	0-20 (Mg ha^{-1})	0-10 (%)	Fina (%)[b]	Planta (g)	Florescimento	Colheita
Trigo	6,34	5,98	0,94	1,24	75	100	0,39	0,20 ± 0,03	0,21 ± 0,02
Triticale	4,26	3,84	1,29	1,40	92	100	0,92	0,33 ± 0,04	0,37 ± 0,04
LSD[c]	1,35	0,97	0,40	0,43	6	9	2,73	0,05	0,07

[a] Total de MS de raízes (0-20 cm). Médias ± desvio padrão (DP).
[b] Raízes finas (1<Ø<2 mm).
[c] Least Significant Difference (P<0,05).

Tabela 4: Conteúdo de C e N totais (g kg^{-1}), total acumulado (kg ha^{-1}) e partição do C e N na MS com os resíduos da parte aérea (PA) e raízes (R) das espécies de culturas anuais.

(continua)

Espécies	C total		N total		C:N		C acumulado[a]		N acumulado[a]		R/PA[b]	
	R[c]	PA	R[c]	PA	R	PA	R	PA	R	PA	C	N
Leguminosas												
Crotalária juncea	463	464	10,6	16,0	44	29	406	4129	9,3	142,8	0,10 ± 0,02	0,06 ± 0,01
Crotalária spectabilis	474	483	8,0	24,3	59	20	279	3474	4,7	174,8	0,08 ± 0,01	0,03 ± 0,02
Feijão	455	460	13,1	18,9	35	24	332	1611	9,5	66,1	0,15 ± 0,03	0,11 ± 0,02
Feijão de porco	460	478	13,6	40,7	34	12	247	3822	7,3	325,0	0,06 ± 0,01	0,02 ± 0,03
Guandu anão	465	484	11,8	21,4	39	23	670	6288	17,2	278,0	0,11 ± 0,03	0,06 ± 0,01
Mucuna cinza	465	480	25,0	31,4	19	15	310	2754	16,6	180,0	0,11 ± 0,02	0,09 ± 0,02
Soja	462	474	14,6	12,3	32	39	374	3490	11,7	90,3	0,08 ± 0,02	0,10 ± 0,03
Tremoço azul	460	476	11,2	35,4	41	14	392	2637	9,5	195,7	0,15 ± 0,04	0,05 ± 0,01
Tremoço nativo	467	473	11,8	29,0	40	16	369	2609	9,2	159,8	0,14 ± 0,02	0,06 ± 0,01
Ervilha	464	470	37,3	43,2	13	11	309	2554	24,7	233,4	0,12 ± 0,02	0,11 ± 0,02
Ervilhaca	469	480	37,9	38,3	12	13	624	1758	50,4	140,7	0,36 ± 0,05	0,36 ± 0,06
Não leguminosas												
Girassol	469	464	5,2	10,4	90	45	864	4264	9,6	95,6	0,15 ± 0,02	0,08 ± 0,01
Mamona	470	481	8,8	19,6	54	25	248	1494	4,6	61,2	0,16 ± 0,04	0,07 ± 0,02
Milho	481	464	13,9	4,4	35	105	654	3039	18,9	28,9	0,18 ± 0,02	0,56 ± 0,09
Sorgo	464	480	12,0	17,9	39	27	856	3662	22,1	136,5	0,22 ± 0,03	0,15 ± 0,02
Milheto	448	460	11,4	10,3	40	45	720	10334	18,2	232,1	0,07 ± 0,01	0,08 ± 0,01
Canola	452	479	6,2	30,0	73	16	379	1139	5,2	71,1	0,29 ± 0,07	0,06 ± 0,01
Nabo forrageiro	462	459	11,4	17,4	41	26	397	3129	9,8	118,7	0,13 ± 0,02	0,08 ± 0,02
Arroz	444	459	13,0	9,7	34	47	1027	4558	30,2	85,6	0,19 ± 0,02	0,30 ± 0,03
Aveia amarela	446	467	12,5	12,7	36	37	812	2592	22,7	70,6	0,27 ± 0,05	0,28 ± 0,05
Aveia branca	467	474	13,3	12,1	35	39	635	2008	18,1	50,9	0,28 ± 0,03	0,31 ± 0,04
Aveia preta	461	478	11,7	13,1	40	37	855	3464	21,6	94,8	0,22 ± 0,04	0,21 ± 0,03
Azevém	458	464	8,2	4,7	56	98	529	2269	9,4	23,2	0,21 ± 0,02	0,36 ± 0,03
Centeio	424	474	12,1	4,7	35	102	579	2400	16,6	23,5	0,21 ± 0,03	0,61 ± 0,13
Cevada	490	464	14,7	5,6	33	83	492	2227	14,8	26,8	0,19 ± 0,02	0,46 ± 0,07

Tabela 4: Conteúdo de C e N totais (g kg^{-1}), total acumulado (kg ha^{-1}) e partição do C e N na MS com os resíduos da parte aérea (PA) e raízes (R) das espécies de culturas anuais.

(conclusão)

Espécies	C total		N total		C:N		C acumulado[a]		N acumulado[a]		R/PA[b]	
	R[c]	PA	R[c]	PA	R	PA	R	PA	R	PA	C	N
Trigo	464	466	11,0	5,2	42	90	576	2790	13,7	31,0	0,20 ± 0,03	0,42 ± 0,05
Triticale	459	468	18,0	4,9	26	95	645	1794	25,3	18,8	0,32 ± 0,03	1,21 ± 0,15
LSD[d]	19,7	8,5	1,0	1,4	2,1	2,4	190,6	546,7	5,3	23,7	0,05	0,08

[a] Acúmulo na MS de todas as raízes e parte aérea das plantas de cobertura no florescimento. Acúmulo nos resíduos de colheita da parte aérea das culturas principais (tabela 2).
[b] Partição do C e N nas plantas considerando a MS total de raízes e parte aérea no florescimento. Médias ± desvio padrão (DP).
[c] Acúmulo proporcional a MS de raízes grossas e finas ou somente nas raízes finas de acordo com cada espécie (Tabela 3).
[d] Least Significant Difference (P<0,05).

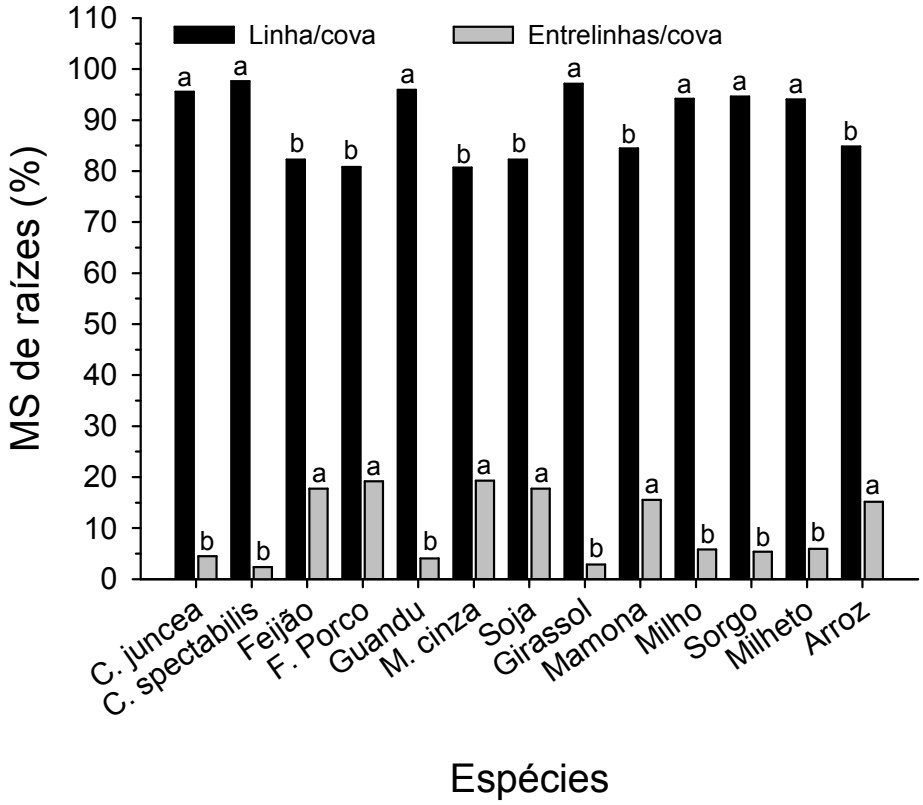

Figura 1: Distribuição da MS no solo em relação à linha de semeadura/cova para espécies de primavera/verão na camada de 0-10 cm. Culturas: C. juncea = Crotalária juncea; C. spectabilis = Crotalária spectabilis; F. porco = Feijão de porco; Guandu = Guandu anão; M. cinza = Mucuna cinza. Médias seguidas das mesmas letras maiúsculas ou minúsculas não diferem estatisticamente entre si pelo teste LSD (P<0,05).

3 ARTIGO II – CARBON MINERALIZATION IN SOIL OF ROOTS FROM TWENTY CROP SPECIES, AS AFFECTED BY THEIR CHEMICAL COMPOSITION AND BOTANICAL FAMILY[2]

3.1 Abstract

Background and aims: Our objective was to relate chemical composition of roots of a wide range of annual crops to root decomposition, so as to assess roots potential contribution to soil carbon (C).

Methods: Roots from 20 different crops and 4 botanical families, collected under field conditions were incubated in soil for 120 days at 25°C. The initial chemical composition of roots was determined. The C mineralization was assessed by the continuous measurement of CO_2 release and using single exponential model. Principal component analysis (PCA) was used to explore qualitative pattern in root quality and decomposition.

Results: PCA analysis showed that chemical characteristics (traits) differentiated plant families. The mineralization of root C varied greatly in terms of kinetics and in the total amount of C mineralized (36% to 59% of added C). Mineralization constant (*k* value) was negatively correlated with hemicelluloses and positively with N content. Poaceae roots that combined high hemicelluloses content, low cellulose and low total N, showed low degradation rate and cumulative C mineralization.

Conclusions: The chemical composition of roots, as for the above-ground parts of plants, can correctly predict their rate of decomposition in soils. The taxonomic affiliation enhances the understanding of the chemical determinants of quality of roots.

Keywords: Carbon mineralization; Chemical composition; Decomposition rate; Litter quality; Root; Trait

3.2 Introduction

[2] Artigo elaborado de acordo com as normas da revista Plant and Soil (Anexo A). Artigo aceito para publicação.

The decomposition of plant residues is a key process in terrestrial agro-ecosystems. However, the decomposition of plant residues has been much better studied for above-ground than for below-ground components (Zhang et al. 2008). Nonetheless, given the role of cropped soils in C sequestration, the dynamics of root decomposition and C stabilization in soil has become a major environmental challenge and received increasing attention during the last decade (Abiven et al. 2005, Rasse et al. 2005; Aulen et al. 2012). Within a given management system, many of the factors (e.g., climate and tillage) that influence SOC turnover will be similar. The quantity of residue returned to the soil influences the soil organic matter content, but quality differences among residues become important when other factors are held constant. According to many authors (Heal et al. 1997; Trinsoutrot et al. 2000; Bertrand et al. 2006), the prediction of C mineralization as a function of chemical quality is complex for plant tissues because of the numerous interactions between plant components (e.g., lignin, polysaccharides, polyphenols, and soluble fractions). Roots, which are rich in secondary cell walls, typically contain more lignified cells than aerial plant parts (De Neergaard et al. 2002; Bertrand et al. 2006; Carrera et al. 2008).

Because most studies investigating plant root decomposition have been restricted to a limited number of plant species and have utilized various incubation protocols (e.g., temperature, humidity, N available), it is difficult to establish a generic relationship between the chemical features of roots and C decomposition in soil for varied species. In natural ecosystems, it has been shown that root morphology and chemistry differ widely between species (Aulen et al. 2012; Picon-cochard et al. 2012) and roots “traits values” are used to study interspecific variation in roots decomposition. For example, it was shown that Poaceae, with high cellulose concentration and low concentrations of soluble compounds decomposed more slowly than Asteraceae and Fabaceae (Birouste et al. 2012).

Therefore, the first objective of this study was to assess the chemical composition of roots in a large range of annual crop species and to investigate how their chemical composition affects their potential mineralization in soil. The second objective of this study was to examine whether the decomposition rates of roots of annual crops differ between taxonomic groups. In this study, we selected twenty crops from agricultural systems in Brazil with varied agronomic uses (main and cover crops) and in which we expected divergent chemical compositions. We compared the potential decomposition rates of roots - measured under standard conditions - by controlling other

parameters to avoid confounding effects (particularly the availability of inorganic soil N). We hypothesized that the botanical family of the different crops dictates their chemical features and has a significant impact on their decomposition in soil.

3.3 Materials and methods

3.3.1 Plant roots

Twenty representative species of plants (main crops and cover crops) of agricultural systems in Brazil were studied (Table 1). The plants selected included nine Poaceae (Gramineae), eight Fabaceae (legumes), two Brassicaceae, and one Asteraceae. The plants were cultivated during the autumn/winter (9 species) and spring/summer (11 species) in a Typic Hapludalf soil under a no-till system in the experimental area of the Soil Department (29°41′ S, 53°48′ W; approximately 90 m elevation) of the Federal University of Santa Maria in the state of Rio Grande do Sul, Brazil. The region has a subtropical climate, with a mean annual precipitation of 1,686 mm and a mean air temperature of 19.3ºC. For the previous 12 years, the experimental site had been cultivated using a no-till system. All the crops were given appropriate management according to the technical recommendations for the area. The roots of the plants were collected using an excavation method at the flowering stage in the 0-10 cm-layer of micro-plots (with areas of 0.09 to 0.36 m^2, depending on the type of crop), with 3 field replicates. The sampling stage was chosen as the most representative time for the root system mass and composition at the start of senescence during the growing season (e.g., Amos and Walters 2006).

The roots were manually separated from the soil under running water over a sieve with 1-mm-diameter openings; the nodules were removed for the Fabaceae species plants, and the roots were dried at 40°C. The roots were separated by diameter (Ø) into coarse (Ø≥2 mm) and fine (1<Ø<2 mm) fractions to determine the proportions of these fractions for each plant species (Table 1). Coarse roots were cut into pieces approximately 0.3 cm thick. All the roots (fine) and root pieces (coarse) were then cut into pieces 1 cm in length for incubation. A sub-sample of each type of root was also dried at 65°C and finely ground (<1 mm) for the chemical analyses.

3.3.2 Chemical analyses

The total organic carbon (C) and total N (N) contents of the roots were determined from finely ground sub-samples dried at 65ºC using an elemental autoanalyzer (FlashEA 1112, Thermo Finnigan, Milan, Italy). A proximate analysis using the Van Soest method was performed using sub-samples of ground roots pre-dried at 40°C. The soluble (SOL), cellulose (CEL), hemicellulose (HEM), and lignin (LIG) fractions of the roots were determined by proximate analysis (Van Soest 1963). Neutral digestion was performed with 0.3 g of roots and 30 ml of neutral detergent solution. For acid digestion, 0.6 g of roots and 60 ml of detergent solution acid were used. The digestions were performed by boiling the material in the digester block at 150ºC for 1 hour. After digestion, the samples were filtered by vacuum suction using a filter crucible and then washed with hot distilled water (90°C) and acetone (30–40 ml). The fibers were dried at 105°C for 12 hours. The SOL content was determined from the difference in the weight before and after neutral digestion. The HEM content was determined from the difference between the percentage of total neutral fiber and the percentage of acid detergent fiber. The CEL content of the acid fiber was obtained after digestion with 12 M H_2SO_4 for 3 hours; the material remaining after quantification of the CEL content was burned in a muffle furnace at 500ºC for 3 hours to determine the LIG content. The water-soluble organic carbon (Csw), and water-soluble total nitrogen (Nsw) was extracted using 0.5 g of finely ground roots pre-dried at 40°C. The roots were placed in a 60-ml snap cap with distilled water (20°C) and mechanically stirred for 30 minutes. After mixing, the material was filtered (Whatman n°5), and the contents of Csw and Nsw in the filtrate were determined by expressing at 65°C. The quantification of soluble polyphenols (POL) was made with 0.75g of finely ground roots pre-dried at 40°C. POL extraction was made with aqueous methanol (1:1) in a water bath at 80°C for 1 hour (Tian et al. 1995). After extraction, the aqueous solution was filtered (Whatman n°2). After addition of the Follin-Denis reagent, POL content was determined by colorimetry (absorbance 760nm) (King and Health 1967). All the analyses were performed with three replicates and the results are shown in Table 2.

3.3.3 Soil, treatments, and experimental conditions

The soil used in the incubation experiment was a Typic Hapludalf (USDA classification) collected from the 0-10-cm layer in the same area as the plants. This soil contains 120 g kg^{-1} clay, 280 g kg^{-1} silt, 600 g kg^{-1} sand, 8.7 g kg^{-1} organic C, and 0.9 g kg^{-1} total N and has a pH (soil H_2O) of 5.4. Fresh soil samples were sieved to 4 mm and then cleaned manually to remove the visible crop residues. Potassium nitrate (KNO_3) was added to obtain a final concentration of 77 mg N kg^{-1} of soil to prevent limited decomposition due to N availability (Recous et al. 1995). The concentration of the added N solution was calculated to achieve a soil moisture content of 80% of field capacity. The soil was pre-incubated in plastic bags at 25°C for 5 days.

The experiment consisted of an incubation conducted for 120 days in the dark at 25±1°C to measure C mineralization of the roots. The 21 treatments consisted of a non-amended soil (as a control) and soil + roots for the 20 plant species listed in Table 1. The treatments were arranged as a completely randomized design, and each treatment was replicated three times. For each replicate, a sub-sample of 134 g soil at 13.8% moisture (equivalent to 80% of field capacity) was obtained, to which particles of root were added at the rate of 2.5 g DM (dry matter) per kg of dry soil (equal to an incorporation rate of 1.53 Mg DM ha^{-1}). This was equivalent to the addition of 874 (wheat) to 1,085 (soybean) mg C kg^{-1} of dry soil and 12 (sunflower) to 86 (vetch) mg N kg^{-1} of dry soil. First, for each replicate, a sub-sample of 67 g moist soil + root particles was placed in a 110-ml cylindrical acrylic pot (5.0 cm in diameter and 5.0 cm in height) and compressed to a height of 2.5 cm. Then a second subsample of 67 g of soil + roots particles was placed in the same acrylic pot and compressed to a total height of 5cm. Thus, the soil in each pot reached a final bulk density of 1.2 g cm^{-3}. Each acrylic pot was placed in a 1,000-ml glass jar prior to incubation.

3.3.4 Analytical procedures

The C mineralization of the roots was assessed by quantifying CO_2 release. The CO_2 produced in the soil was trapped in 10 ml of 1 M NaOH in a beaker placed inside each glass jar. The carbonate trapped in the NaOH was precipitated with a $BaCl_2$ solution in excess of 2 M, and the remaining NaOH was titrated with 1 M HCl. Titrations were performed at 2, 4, 7, 10, 14, 21, 28, 35, 50, 70, 90, and 120 days and the NaOH beakers were changed at each sampling time. At all sampling times, the jars were

aerated for 10 minutes to renew the internal atmosphere, and the soil water content was checked by weighing and was adjusted as necessary. The apparent C mineralization of the roots was calculated by subtracting the amounts of CO_2-C evolved from the control treatment from the amounts of CO_2-C evolved from the amended treatments. Apparent mineralization assumes that there is no effect of root addition on soil C mineralization (no priming effect) or that this effect is similar, regardless of the type of roots added.

3.3.5 Data and statistical analysis

Data of kinetics of mineralization and of cumulative mineralized C measured over 120 days were analyzed by one-way analysis of variance. Scott-Knott test ($P<0.05$) were used to determine where significant differences occurred. All data were analyzed using STATISTICA® statistical software (version 7.0).

To get a quantitative measure of the relative importance of the initial chemical characteristics of roots in determining root mineralization, we first calculated C mineralization using an exponential equation, according to Jung et al. (2011):

$$Cmin = C_0 (1 - e^{-k\,\mathrm{day}}) \qquad (1)$$

with *Cmin* is the amount of mineralized carbon, C_0 is a potentially mineralizable C pool, k is a mineralization constant, and *day* is the incubation period.

Stepwise multiple regression analysis was then used to determine which combinations of chemical variables best explained the variations in C_0 and k. Only those variables that were found to be significant at $P<0.05$ were retained in the regressions. Regressions were performed with all available chemical variables and with the variables obtained by Van Soest analysis, only.

A principal component analysis (PCA) was performed to address the question of what are the chemical traits that differentiate plant families, according to Aulen et al. (2012). PCA was done on the correlation matrix obtained from the results of the proximate analysis (i.e., SOL, CEL, HEM, and LIG), C, N, water-soluble fractions (i.e., Csw, and Nsw) and POL. The potentially mineralizable C pool (C_0), and the mineralization constant (k) were added as supplementary variables (i.e., not included in the ordination of species) to analyze relationships with chemical characteristics. The

PCA produced an ordination of the species and the chemical composition of the roots, which were plotted in one and two dimensions, respectively, based on the scores of the first two principal components (PCs). A correlation circle was computed to assess the importance of each chemical component in the PC axes. We considered *r* values (i.e., correlation coefficient) as significant contributor when $r \geq 0.5$ (e.g., Guénon et al. 2011).

3.4 Results

3.4.1 Chemical compositions of roots

The chemical compositions of the roots studied are summarized in Table 2 and were analyzed by a principal component analysis (PCA) to identify the most significant differences among the species (Fig. 1). The C contents ranged from 424 g kg^{-1} (rye) to 490 g kg^{-1} (barley), and the N contents ranged from 5.2 g kg^{-1} (sunflower) to 37.9 g kg^{-1} (vetch), resulting in C:N ratios ranging from 12 to 90 (Table 2). The LIG contents of the roots ranged from 62 g kg^{-1} (ryegrass) to 188 g kg^{-1} (soybean) with, on average, lower LIG contents in the roots of Poaceae species plants than in the roots of plants from other families. The roots of dicotyledonous species (mainly Fabaceae) exhibited high variability in their chemical compositions, with, on average, higher SOL, CEL, LIG, and total N contents than the roots of the species from the Poaceae family. As a result, this latter group exhibited low variability in composition among different species and was characterized by higher HEM contents (Table 2).

PCA showed that the first two principal components (PC) accounted for 71% of the total variance of the data (Fig. 1). PC 1 explained 50% of the variance of the data and discriminated clearly the Fabaceae species with mucuna (7), vetch (12) and pea (16) in the negative part (Fig. 1a) significantly correlated with the soluble fractions (Van Soest and water-soluble), POL and total N contents (in the range $r = -0.91$ to -0.93) (Fig. 1b). Other Fabaceae, Asteraceae, and Brassicaceae species were in the positive part, and most strongly correlated with the CEL ($r=0.73$) (Fig. 1a, b). PC 2 explained 21% of the variance of the data, and identified the Poaceae species mostly in the positive part of PCA (Fig. 1a), as significantly correlated with the HEM content ($r=0.91$) (Fig. 1b). SOL, N, Csw, Nsw and POL appeared strongly correlated to each other which mainly reflects the chemical composition of the Fabaceae species 7, 12 and 16.

3.4.2 Carbon mineralization

The cumulative C mineralization from roots, expressed as the proportion of added C, varied widely among the treatments, both in terms of kinetics and of total C mineralized at day 120 (Fig. 2a; Table 3). At the end of the incubation period (120 days), the cumulative C mineralized varied from 36 ± 2.5% added C (barley, number 19) to 59 ± 3.5% added C (sunflower, number 4). The species can be grouped into three groups based on statistical differences in the amounts of cumulative C mineralized measured during 120 days. In the first and second groups, with three species each, mineralized C ranged from 56.9 to 59.3% added C and 48.5% to 53.1% added C, respectively; in the third group, 14 species showed mineralized C values ranging from 35.8 to 44.4% added C. However, the kinetics of mineralization showed strong differences between species as shown, as example, with species 1, 10, 12 and 19 (Fig. 2b). The roots of species 10, 12 and 19 had no significant differences in their total C mineralized, but had very different kinetics of mineralization.

In general the roots of the Fabaceae species plants exhibited higher levels of cumulative C mineralization (ranging from 38% for showy rattlebox to 58% for bean) than the roots of Poaceae species plants (ranging from 36% for barley to 49% for sorghum). The roots of the single Asteraceae species studied (sunflower, number 4) exhibited the highest cumulative C mineralization (59%), whereas the two Brassicaceae species (numbers 14 and 18) were in the low and high range (39 and 53%, respectively).

The potentially mineralizable C pool (C_0) calculated with a simple exponential decay function using the cumulative C mineralized (Fig. 2a) ranged from 33.8 to 58.7% added C and was close to the measured cumulative C mineralized during 120 days (Table 3). The mineralization constant (k) of the C_0 differed among roots (Table 3) and ranged from 0.024 for millet root (8) to 0.134 for vetch root (12).

3.4.3 Relationships between the root chemical composition and C mineralization

The relationships between the decomposition constants of C mineralized (k; Table 3) and the initial root chemical composition, using all chemical characteristics and analyzed by stepwise regression with standardized coefficients was k= -0.29 HEM

+ 0.74 N, r^2=0.74, P<0.001. The regression using only data from proximate analysis (SOL, HEM, CEL and LIG) was k= -0.75 HEM -0.75 CEL, r^2=0.72, P<0.001. For C_0, the corresponding equations were C_0= 0.72 CEL + 0.54 Csw, r^2=0.56, P<0.001 (all chemical data) and C_0= 0.55 CEL, r^2=0.30, P<0.001 (proximate analysis). Using the PCA approach, the mineralization constant (*k)* was positively correlated with POL, SOL, N and soluble fractions (Fig. 1b), and negatively correlated with CEL and HEM. The potential mineralization pool (C_0) was positively correlated CEL and LIG, and negatively correlated with HEM.

3.5 Discussion

3.5.1 Chemical composition of roots

Many plants used in crop rotations differ widely with regard to the chemical composition of their tissues (Abiven et al. 2005; Silver and Miya 2001), and the roots of these plants may contribute differently to soil C inputs (Rasse et al. 2005; Johnson et al. 2007; Kong and Six 2010). The results of the present study confirmed our hypothesis that there is a large variation in the root biochemical composition among plant species grown under similar conditions, which can be partly explained by the fact that the plants belong to different botanical families. Indeed, this has also been clearly shown with plant species from natural ecosystems, and identified as interspecific variations in functional traits (Roumet et al. 2008; Aulen et al. 2012; Picon-Cochard et al. 2012). The roots of the Fabaceae (legumes), Asteraceae, and Brassicaceae families, which are all in the dicotyledons, were characterized by large variations in composition among species and by their higher, on average, CEL and LIG fractions compared to Poaceae roots. Conversely, the proportion of HEM in the roots of Poaceae family was higher than for other species, and the differences in composition among Poaceae species were lower. These differences reflect the different types of root systems with a greater proportion of coarse roots with Fabaceae and Brassicaceae species compared to Poaceae species, which implies a relatively higher amount of plant cell wall material. Although maize, millet, and sorghum also have coarse roots, their LIG contents were found to be in the range of other Poaceae species, suggesting that taxonomical affiliation prevails over root morphology. Roumet et al. (2008) reported that the root tissues of Fabaceae species

are characterized by higher total N than those of Poaceae species, which is consistent with our results; based on their study of a range of functional traits of roots, these authors concluded that Poaceae and Fabaceae differ for the entire set of traits examined. However, the interspecific variation did not explain all differences, and it is noticeable that within the Fabaceae family, pea (16) vetch (12) and gray mucuna (7) strongly differed from other species without any identified explanation associated to crop management.

The average LIG content of the roots of the 20 species of plants considered in the present study (119 g LIG kg^{-1} DM) was lower than the values reported in most published studies, such as that of Abiven et al. (2005), who reported values in the range of 160 to 260 g LIG kg^{-1} DM. Using a global dataset, Silver and Miya (2001) also measured high LIG contents in the roots of graminoid (Poaceae), broadleaf, and conifer (180 to 270 g LIG kg^{-1} DM). In contrast, Lindedam et al. (2009) measured lower LIG contents in grass and clover roots (110 g kg^{-1}), consistent with our results. The relatively low LIG content of the roots used in our study might be explained by the fact that they were collected at the flowering stage. Bertrand et al. (2009) showed that the content of the cell wall material of wheat internodes increased by 85% from the flowering stage to physiological maturity, and Abiven et al. (2011) observed that the LIG contents of maize and wheat roots increased substantially between the flowering and maturity stages, with a larger increase in maize roots (i.e., by a factor of 3.3). In addition, Picon-Cochard et al. (2012) observed that the LIG contents of the roots of 13 grass species varied over the growing season. Indeed, change in the chemical composition of roots with the season or plant development stage is an important factor to consider when comparing families or species of plants or using data from the literature. These findings suggest that our data may represent the quality of the root system of annual crops at a time at which root turnover is often considered to begin (Rasse et al. 2005) but most likely underestimate the lignin content of the roots at crop maturity. However, a comparison of our data and that from the literature cited above, showed that the pattern observed for the chemical quality of the roots as a function of plant species and botanical family was consistent.

3.5.2 Effect of chemical composition on C mineralization

Both the cumulative C mineralization after 120 days of incubation and the kinetics of C mineralization varied greatly among the species. The range of cumulative C mineralization of the root C (from 36 to 59% of added C) and the differences between Fabaceae and Poaceae species were consistent with the observations in other studies conducted under similar experimental conditions (Trinsoutrot et al. 2000; De Neergaard et al. 2002; Abiven et al. 2005). De Neergaard et al. (2002) also detected a large difference in the cumulative C mineralization between white clover roots (Fabaceae) and perennial ryegrass roots (Poaceae), with the clover roots mineralizing approximately 50% more C than ryegrass after 94 days of incubation. Lindedam et al. (2009) observed that the extent of decomposition of clover roots was double that of fescue grass roots after 118 days of incubation.

We assessed prediction of root mineralization as a function of chemical quality by different ways either related to the size of the C pool mineralized (measured and modeled) or to the rates of mineralization (measured and modeled). The roots showed mineralization kinetics very different, but ultimately they are classified in only three statistically different groups, according to their cumulative total mineralization at day 120. The correlations shown with PCA analysis indicated that total N, total soluble polyphenols and soluble fractions (Van Soest, water soluble N and C) were correlated with *k*. The analysis made with multiple regressions indicated that the best plant characteristics to predict *k* were hemicelluloses and total N concentrations. As discussed by Aulen et al. (2012), the effect of N on litter decomposition rate is controversial. Two factors are involved into the N-controlled mechanisms: the intrinsic tissue N content and the extrinsic soil inorganic N nutrient availability which may control decomposition when overall N availability is limiting decomposers activity. In our study, we added inorganic N in soil prior to the incubation, to avoid a potential limitation of decomposition by the availability of soil N and to univocally examine the effects of the plant tissues composition. The results suggest that the available root N (total soluble organic and inorganic N represented, on average, 47% and 7% of the total N, respectively in roots) favored the fast decomposition, particularly for root residues which also had high soluble C content. Conversely, polyphenols content was not relevant to predict mineralization. Indeed high polyphenols content was associated to high N content in the Fabaceae roots, as expected in leguminous plants (Constantinides and Fownes, 1994).

Plant residues consist of the soluble and insoluble fractions (cell wall). Cell walls consist mainly of insoluble polymers such as cellulose, hemicelluloses and lignin, which form a complex, chemical network that influences biological degradation (Chesson 1988; Bertrand et al. 2006). Our results did not show that lignin content was relevant for predicting the mineralization constant (k) and the mineralizable C pool (C_0). Indeed lignin control of decomposition rates is not always confirmed. Silver and Miya (2001) looking at global pattern of root decomposition, also found that lignin explained only small proportion of the pattern observed. In the work of Trinsoutrot et al. (2000) with mineralization data from 47 different crop residues, lignin content did not appear as a predictor of the mineralization constant k. Jensen et al. (2005) using 249 different above-ground residues from crops, found that holocellulose (hemicelluloses + cellulose) was the single factor that explain the variability of C mineralization. Recently, finer scale residue chemical characteristics related to hemicellulose substitution level (i.e., arabinose to xylose ratio), and interactions between cellulose, hemicellulose and lignin have provided new insights to chemical controls on decomposition (Machinet et al. 2009, 2011). These studies demonstrated that C mineralization of maize roots in soil was described by both cell wall polymer content, cross linking agents (phenolic acids) between hemicellulose and lignin, and the substitution level of hemicelluloses.

3.6 Conclusions

Our results with a large database with roots of annual plants, confirm the central role of hemicellulose and cellulose in the ability of roots to biodegradation. Several studies have shown that the contribution of roots to soil organic C is 2.4 (Rasse et al. 2005) to 13 times higher than the contribution of shoots (Kong and Six 2010). According to Aulen et al. (2012), the decomposition of fine roots involves constant and intimate interaction at a very fine spatial scale between roots and their surrounding rhizosphere. The fact that the Poaceae species (Gramineae) plants considered in this study had higher proportions of fine roots and the lowest levels of cumulative mineralized C suggests that fine roots of graminae could make an important contribution to C storage in soil. A few other crop species from the Fabaceae family, used in this study, had similar characteristics. Therefore, the appropriate management of crop rotation with species belonging to Poaceae and Fabaceae, intercropped with cover

crops, may represent an important mechanism for the combined stabilization of C in soil and the recycling of nutrients for crops.

Acknowledgments

This work was supported by the Brazilian government through the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). The bilateral Brazilian and French collaboration was funded under the Program CNPq - Ciência sem Fronteiras (Process Number 401724/2012-3) - and by the INRA (the Environment & Agronomy Division) during M. Redin's residence at UMR FARE in Reims, France. The authors thank anonymous referees for their constructive comments on the previous version of this work.

3.7 References

Abiven S, Recous S, Reyes V, Oliver R (2005) Mineralisation of C and N from root, stem and leaf residues in soil and role of their biochemical quality. Biol Fertil Soils 42:119-128

Abiven S, Heim A, Schmidt MWI (2011) Lignin content and chemical characteristics in maize and wheat vary between plant organs and growth stages: consequences for assessing lignin dynamics in soil. Plant Soil 343:369-378

Amos B, Walters DT (2006) Maize root biomass and net rhizodeposited carbon: An analysis of the literature. Soil Sci Soc Am J 70:1489-1503

Aulen M, Shipley B, Bradley R (2012) Prediction of in situ root decomposition rates in an interspecific context from chemical and morphological traits. Ann Bot 109:287-297

Bertrand I, Chabbert B, Kurek B, Recous S (2006) Can the biochemical features and histology of wheat residues explain their decomposition in soil? Plant Soil 281:291-307

Bertrand I, Prevot M, Brigitte C (2009) Soil decomposition of wheat internodes of different maturity stages: Relative impact of the soluble and structural fractions. Bioresour Technol 100:155-163

Birouste M, Kazakou E, Blanchard A, Roumet C (2012) Plant traits and decomposition: are the relationships for roots comparable to those for leaves? Ann Bot 109: 463-472

Carrera AL, Bertiller MB, Larreguy C (2008) Leaf litterfall, fine-root production, and decomposition in shrublands with different canopy structure induced by grazing in the Patagonian Monte, Argentina. Plant Soil 311:39-50

Chesson A (1988) Lignin-polysaccharide complexes of the plant cell wall and their effect on microbial degradation in the rumen. Anim Feed Sci Technol 21:219-228

Constantinides M, Fownes JH (1994) Nitrogen mineralization dynamics of soil mineral N from leaves and litter of tropical plants: Relationship to nitrogen, lignin and soluble polyphenol concentrations. Soil Biol Biochem 26:49-55

De Neergaard A, Hauggaard-Nielsen H, Jensen LS, Magid J (2002) Decomposition of white clover (*Trifolium repens*) and ryegrass (*Lolium perenne*) components: C and N dynamics simulated with the DAISY soil organic matter submodel. Eur J Agron 16:43-55

Guénon R, Vennetier M, Pailler A, Dupuy N, Roussos S, Gros R (2013) Trends in recovery of Mediterranean soil chemical properties and microbial activities after infrequent and frequent wildfires. Land Degrad Dev 24:115-128

Heal OW, Anderson JM, Swift MJ (1997) Plant litter quality and decomposition: an historical overview. In: Cadish G, Giller KE (eds) Driven by nature: plant litter quality and decomposition. CAB International, Wallingford, pp 3-30

Jensen LS, Salo T, Palmason F, Breland TA, Henriksen TM, Stenberg B, Pedersen A, Lundström C, Esala M (2005) Influence of biochemical quality on C and N mineralisation from a broad variety of plant materials in soil. Plant Soil 273:307-326

Johnson JMF, Barbour NW, Weyers SL (2007) Chemical composition of crop biomass impacts its decomposition. Soil Sci Soc Am J 71:155-162

Jung JY, Lal R, Ussiri DAN (2011) Changes in CO_2, ^{13}C abundance, inorganic nitrogen, β-glucosidase, and oxidative enzyme activities of soil during the decomposition of switchgrass root carbon as affected by inorganic nitrogen additions. Biol Fertil Soils 47:801-813

King HG, Health GW (1967) The chemical analyses of small samples of leaf material and the relationship between the disappearance and composition of leaves. Pedobiologia 7:192-197

Kong AYY, Six J (2010) Tracing cover crop root versus residue carbon into soils from conventional, low-input, and organic cropping systems. Soil Sci Soc Am J 74:1201-1210

Lindedam J, Magid J, Poulsen P, Luxhøi J (2009) Tissue architecture and soil fertility controls on decomposer communities and decomposition of roots. Soil Biol Biochem 41:1040-1049

Machinet GE, Bertrand I, Chabbert B, Watteau F, Villemin G, Recous S (2009) Soil biodegradation of maize root residues: Interaction between chemical characteristics and the presence of colonizing micro-organisms. Soil Biol Biochem 41:1253-1261

Machinet GE, Bertrand I, Barrière Y, Chabbert B, Recous S (2011) Impact of plant cell wall network on biodegradation in soil: Role of lignin composition and phenolic acids in roots from 16 maize genotypes. Soil Biol Biochem 43:1544-1552

Picon-Cochard C, Pilon R, Tarroux E, Pagès L, Robertson J, Dawson L (2012) Effect of species, root branching order and season on the root traits of 13 perennial grass species. Plant Soil 353:47-57

Rasse DP, Rumpel C, Dignac MF (2005) Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant Soil 269:341-356

Recous S, Robin D, Darwis D, Mary B (1995) Soil inorganic N availability: Effect on maize residue decomposition. Soil Biol Biochem 27:1529-1538

Roumet C, Lafont F, Sari M, Warembourg F, Garnier E (2008) Root traits and taxonomic affiliation of nine herbaceous species grown in glasshouse conditions. Plant Soil 312:69-83

Silver WL, Miya RK (2001) Global patterns in root decomposition: comparisons of climate and litter quality effects. Oecologia 129:407-419

Tian G, Brussaard L, Kang BT (1995) An index for assessing the quality of plant residues and evaluating their effects on soil and crop in the sub-humid tropics. Appl Soil Ecol 2:25-32

Trinsoutrot I, Recous S, Bentz B, Linères M, Chèneby D, Nicolardot B (2000a) Biochemical quality of crop residues and carbon and nitrogen mineralization kinetics under nonlimiting nitrogen conditions. Soil Sci Soc Am J 64:918-926

Van Soest PJ (1963) Use of detergents in the analysis of fibrous feeds I: preparation of fiber residues of low nitrogen content. J Assoc Off Ana Chem 46:825-835

Zhang D, Hui D, Luo Y, Zhou G (2008) Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. J Plant Ecol 1:85-93

Table 1: List of the sampled main crops and cover crops and initial proportion of coarse and fine roots.

Binomial nomenclature	Common name	Family	Plant use	% Coarse[a]	% Fine	REF[b]
Helianthus annuus	Sunflower	Asteraceae	Main crop	69 ± 9.6	31 ± 5.8	4
Raphanus sativus oleiferus	Oilseed radish	Brassicaceae	Cover crop	89 ± 4.2	11 ± 7.1	14
Brassica napus oleifera	Oilseed rape	Brassicaceae	Main crop	54 ± 3.9	46 ± 4.8	18
Phaseolus vulgaris	Bean	Fabaceae	Main crop	59 ± 8.5	41 ± 5.3	1
Glycine max	Soybean	Fabaceae	Main crop	55 ± 5.7	45 ± 3.7	2
Crotalaria juncea	Sunn hemp	Fabaceae	Cover crop	74 ± 4.2	26 ± 4.3	5
Canavalia ensiformis	Jack bean	Fabaceae	Cover crop	60 ± 2.4	41 ± 3.2	6
Stizolobium niveum	Gray mucuna	Fabaceae	Cover crop	59 ± 9.0	41 ± 4.8	7
Crotalaria spectabilis	Showy rattlebox	Fabaceae	Cover crop	96 ± 4.1	4 ± 3.9	10
Vicia sativa	Vetch	Fabaceae	Cover crop	0 ± 0.0	100 ± 0.0	12
Pisum arvense	Pea	Fabaceae	Cover crop	0 ± 0.0	100 ± 0.0	16
Zea mays	Maize	Poaceae	Main crop	48 ± 2.1	52 ± 4.6	3
Pennisetum glaucum	Millet	Poaceae	Cover crop	27 ± 2.7	73 ± 3.6	8
Sorghum bicolor	Sorghum	Poaceae	Main crop	58 ± 4.4	42 ± 4.1	9
Avena strigosa	Black oat	Poaceae	Cover crop	0 ± 0.0	100 ± 0.0	11
Triticum aestivum	Wheat	Poaceae	Main crop	0 ± 0.0	100 ± 0.0	13
Secale cereale	Rye	Poaceae	Main crop	0 ± 0.0	100 ± 0.0	15
Triticosecale rimpaui	Triticale	Poaceae	Main crop	0 ± 0.0	100 ± 0.0	17
Hordeum vulgare	Barley	Poaceae	Main crop	0 ± 0.0	100 ± 0.0	19
Lolium multiflorum	Ryegrass	Poaceae	Cover crop	0 ± 0.0	100 ± 0.0	20

[a] Diameter of roots (Ø): Coarse = Ø≥2 mm and fine = 1<Ø<2 mm. Means (n=3) ± standard deviation (SD).
[b] Reference

Table 2: Initial chemical composition of the roots (g kg^{-1} DM): Van Soest fractions, total C and N, water-soluble C and N, and polyphenols content.

Plant (REF)	SOL[a]	HEM	CEL	LIG	C	N	Csw	Nsw	POL
Sunflower (4)	192 ± 3.0	153 ± 8.9	514 ± 3.8	140 ± 6.2	469 ± 4.5	5.2 ± 0.1	71 ± 6.7	1.9 ± 0.5	9.6 ± 0.7
Oilseed radish (14)	328 ± 6.5	144 ± 5.9	402 ± 7.4	126 ± 2.6	462 ± 3.6	11.4 ± 0.1	80 ± 6.0	6.8 ± 1.0	5.7 ± 0.4
Oilseed rape (18)	258 ± 8.1	191 ± 8.5	409 ± 2.3	142 ± 4.9	452 ± 4.9	6.2 ± 0.8	81 ± 8.4	4.3 ± 0.4	3.7 ± 0.1
Bean (1)	238 ± 3.4	189 ± 3.5	449 ± 5.0	124 ± 5.3	459 ± 3.9	13.1 ± 0.2	100 ± 3.3	7.3 ± 0.5	6.6 ± 0.2
Soybean (2)	162 ± 7.3	181 ± 8.9	469 ± 2.4	188 ± 6.5	462 ± 3.2	14.6 ± 0.6	50 ± 2.2	7.5 ± 0.3	7.0 ± 0.4
Sunn hemp (5)	168 ± 1.7	200 ± 8.1	517 ± 9.5	116 ± 4.5	463 ± 2.1	10.6 ± 0.3	69 ± 3.4	2.4 ± 0.1	5.2 ± 0.2
Jack bean (6)	221 ± 2.5	190 ± 7.3	476 ± 1.6	112 ± 8.9	459 ± 3.0	13.6 ± 0.4	73 ± 2.3	3.6 ± 0.1	4.1 ± 0.1
Gray mucuna (7)	393 ± 1.5	104 ± 8.2	361 ± 6.7	141 ± 6.1	465 ± 5.8	25.0 ± 0.2	113 ± 3.8	6.6 ± 0.4	33.6 ± 1.1
Showy rattlebox (10)	91 ± 2.9	217 ± 7.8	507 ± 4.8	185 ± 7.1	474 ± 2.8	8.0 ± 0.3	15 ± 1.8	5.2 ± 0.4	5.2 ± 0.6
Vetch (12)	393 ± 3.4	136 ± 1.6	276 ± 3.0	127 ± 3.7	469 ± 9.2	37.9 ± 0.9	115 ± 14.2	18.7 ± 1.3	24.8 ± 1.2
Pea (16)	414 ± 7.8	202 ± 2.4	279 ± 5.2	105 ± 4.4	464 ± 9.7	37.3 ± 1.8	113 ± 11.8	26.1 ± 2.9	39.5 ± 0.9
Maize (3)	256 ± 2.4	318 ± 2.6	355 ± 8.8	71 ± 7.6	481 ± 4.8	13.9 ± 0.3	83 ± 5.6	10.2 ± 0.8	7.9 ± 0.3
Millet (8)	263 ± 9.0	153 ± 4.5	456 ± 8.4	127 ± 3.7	448 ± 5.2	11.4 ± 0.4	48 ± 3.7	1.0 ± 0.1	10.3 ± 0.7
Sorghum (9)	302 ± 9.9	253 ± 2.0	357 ± 4.1	88 ± 2.6	464 ± 3.8	12.0 ± 0.4	72 ± 3.5	6.4 ± 0.3	17.5 ± 0.3
Black oat (11)	200 ± 2.8	290 ± 2.3	437 ± 4.2	73 ± 4.4	461 ± 9.7	11.7 ± 0.3	57 ± 4.5	3.9 ± 0.6	5.4 ± 0.3
Wheat (13)	195 ± 4.4	291 ± 3.4	371 ± 3.8	142 ± 9.7	464 ± 9.6	11.0 ± 0.6	77 ± 10.8	8.0 ± 1.0	8.6 ± 0.9
Rye (15)	208 ± 5.4	365 ± 4.4	302 ± 8.3	125 ± 2.2	424 ± 9.8	12.1 ± 0.5	40 ± 4.2	6.7 ± 0.4	5.1 ± 0.6
Triticale (17)	262 ± 6.9	322 ± 8.4	329 ± 8.9	88 ± 5.4	459 ± 3.1	18.0 ± 0.6	51 ± 5.3	7.2 ± 0.6	12.0 ± 0.6
Barley (19)	258 ± 4.8	283 ± 3.8	362 ± 8.1	96 ± 9.5	490 ± 9.4	14.7 ± 0.9	42 ± 4.7	5.1 ± 0.6	7.0 ± 0.2
Ryegrass (20)	223 ± 9.0	324 ± 5.8	391 ± 4.4	62 ± 7.5	458 ± 9.6	8.2 ± 0.2	54 ± 3.1	3.1 ± 0.3	8.4 ± 0.2

[a] SOL: Soluble fraction (Van Soest); HEM: Hemicellulose; CEL: Cellulose; LIG: Lignin; C: Total carbon; N: Total nitrogen; Csw: Water-soluble carbon; Nsw: Water-soluble nitrogen; POL: Soluble polyphenols; DM: Dry matter. Means (n=3) ± standard deviation (SD).

Table 3: Observed percentage of residue C mineralization and kinetic coefficients calculated from a simple exponential decay function after 120 days at 25ºC incubation of residue roots in soil.

Plant (REF)	C min measured[a]	C_0[b]	k[c]	R^2[d]
	%	%	day^{-1}	
Sunflower (4)	59.3 a*	58.7	0.038	0.99
Oilseed radish (14)	39.4 c	38.3	0.086	0.99
Oilseed rape (18)	52.7 b	50.1	0.062	0.99
Bean (1)	58.2 a	54.9	0.052	0.99
Soybean (2)	39.2 c	37.2	0.059	0.99
Sunn hemp (5)	53.1 b	50.9	0.038	0.99
Jack bean (6)	56.9 a	53.7	0.071	0.99
Gray mucuna (7)	42.3 c	40.0	0.099	0.98
Showy rattlebox (10)	38.3 c	37.8	0.055	0.99
Vetch (12)	43.1 c	39.5	0.134	0.99
Pea (16)	44.0 c	40.7	0.109	0.98
Maize (3)	43.9 c	41.3	0.057	0.99
Millet (8)	40.3 c	41.9	0.024	0.99
Sorghum (9)	48.5 b	44.0	0.051	0.94
Black oat (11)	44.4 c	44.7	0.027	0.99
Wheat (13)	40.5 c	38.8	0.033	0.98
Rye (15)	40.6 c	38.3	0.046	0.97
Triticale (17)	41.6 c	39.3	0.041	0.95
Barley (19)	35.8 c	33.8	0.065	0.97
Ryegrass (20)	43.0 c	42.3	0.041	0.99

[a] The % of added C mineralized from roots after 120 days.
[b] Potentially mineralizable C pool.
[c] Mineralization constant rate.
[d] Coefficient of determination between measured and predicted values.
* Values with different letters are different by Scott-Knott test (P<0.05).

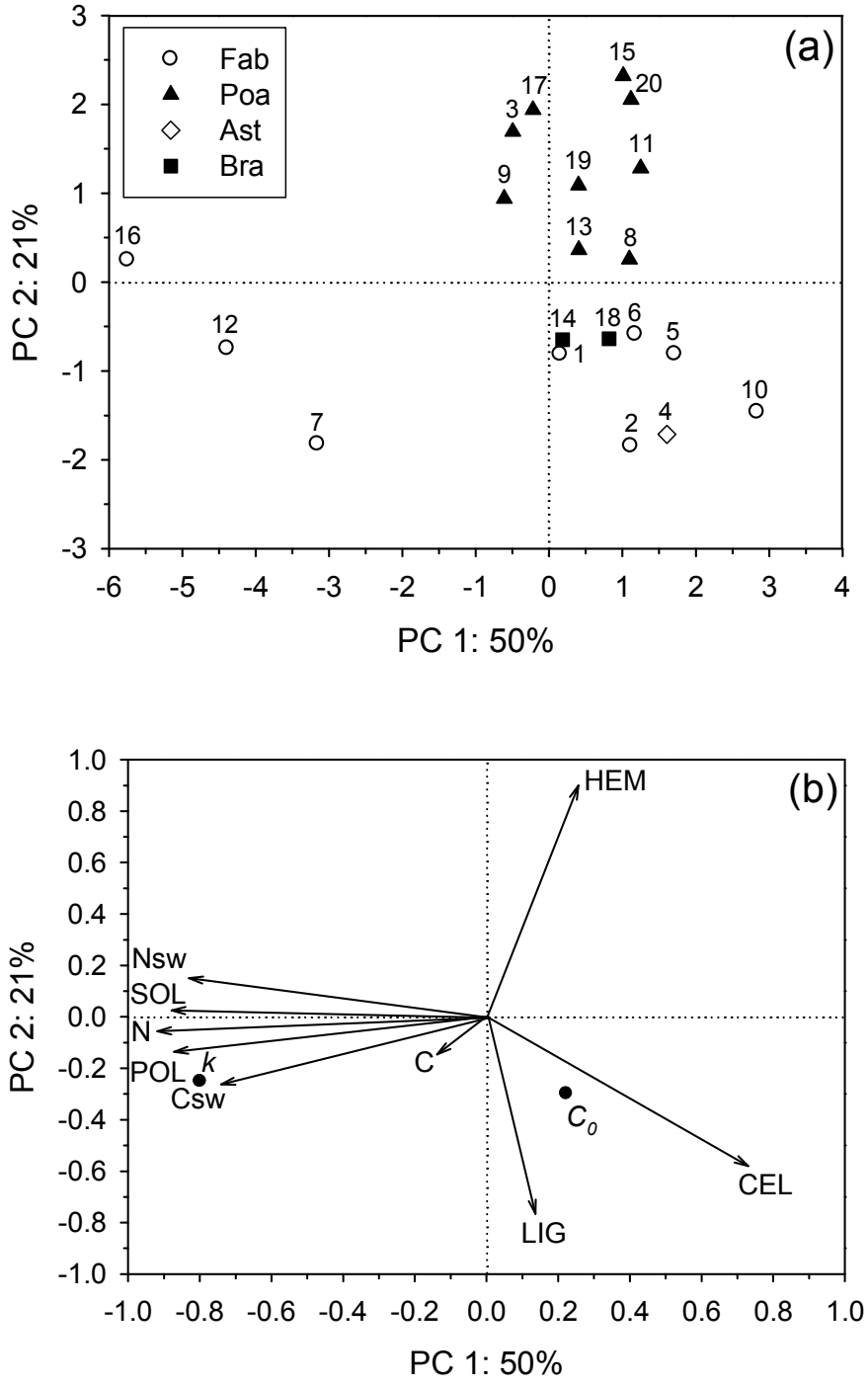

Fig. 1: Factorial map (PC 1 x PC 2) (a) and correlation circle (b) obtained from the principal component analysis (PCA) performed using the data for the chemical composition of 20 root species (i.e., Van Soest fractions, total C, total N, water-soluble fractions and polyphenols). Mineralization constant (k) and mineralizable C pool (C_0) were added as supplementary variables i.e., not included in the ordination of species. The species are identified according to the botanical family. Fab: Fabaceae; Poa: Poaceae; Ast: Asteraceae; Bra: Brassicaceae. The identification numbers of the 20 species studied are provided in Table 1.

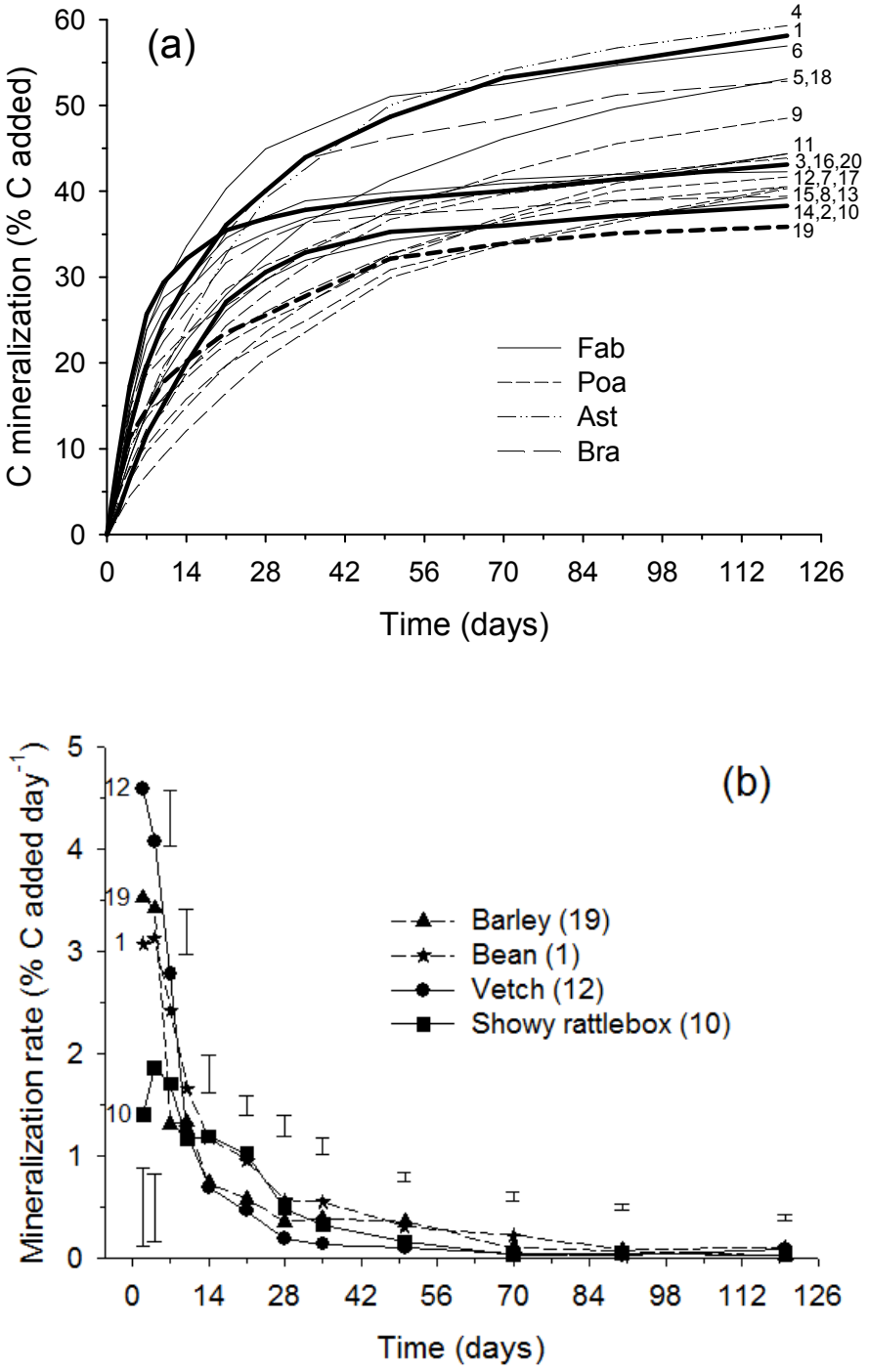

Fig. 2: Cumulative C mineralization of roots of 20 species during incubation in soil during 120 days at 25°C (a) and rates of C mineralization during decomposition for 4 species among the 20 species studied (b). These 4 species are three Fabaceae crops (vetch, 12; bean, 1; showy rattlebox, 10) and 1 Poaceae crop (barley, 19). The data plotted are the means (n=3). The vertical bars indicate the minimum significant difference between the treatments (Tukey's test, P<0.05). Fab: Fabaceae; Poa: Poaceae; Ast: Asteraceae; Bra: Brassicaceae. The identification numbers of the 20 species studied are provided in Table 1.

4 ARTIGO III – THE INFLUENCE OF CHEMICAL COMPOSITION ON THE MINERALIZATION OF PLANT RESIDUES: THE EFFECTS OF LEAF AND STEM MIXTURES FROM 25 CROP SPECIES[3]

4.1 Abstract

The effects of the chemical characteristics of plant litter on C and N mineralization during decomposition were studied using leaf litter, stem litter and mixtures of leaves plus stems for 25 annual crops of five botanical families. The objective was to understand better the decomposition-related interactions (additive or non-additive) between residues of different qualities decomposing together at the soil surface, which is a condition commonly encountered in farming situations without tillage. Residues were characterized chemically and incubated for 120 days at 25°C. The C and N mineralization was measured over time. For mixtures, we quantified the chemical heterogeneity using a coefficient of similarity. The deviation from additivity, i.e., the relative difference between the observed and expected values, was calculated after 120 days. The 25 mixtures represented a wide range of chemical qualities and heterogeneity and exhibited different cumulative C-CO_2 and net N mineralization rates. Litters from Fabaceae (mostly cover crops) included high N and soluble contents, as well as large characteristic differences between stem and leaf litters. Conversely, the litters from Poaceae (Gramineae) were richer in hemicellulose and cellulose, and they decomposed more slowly and exhibited a similar composition between leaf litter and stem litter. Generally, mixed leaf and stem litters exhibited mainly additive effects on C mineralization and N mineralization. Synergistic effects resulted in an average of 9% additional C. For N, an antagonistic effect was observed in most of the Fabaceae mixtures with high N content and high heterogeneity. The decomposition-related interactions in mixtures appeared to be markedly controlled by the degree of heterogeneity of the mixtures, and the results confirm the role of N availability in these interactions.

[3] Artigo elaborado de acordo com as normas da revista Soil Biology and Biochemistry (Anexo B). Artigo submetido.

Keywords: Decomposition; Chemical composition; Functional trait; Leaf; Stem; Litter mixture

4.2 Introduction

No-till systems use a large diversity of crop plant species that directly influence the cycles of carbon (C) and nitrogen (N) in soil (Jensen et al., 2005). In these cropping systems, the crop residues form mulch at the soil surface composed of a mixture of different plant parts (Thippayarugs et al., 2008; Lal et al., 2007). Physical factors (of soil or residue) and biological processes either individually or in combination can drive decomposition, but the intrinsic characteristics, or functional traits, of crop residues are important in controlling their decomposition rate in soils (Trinsoutrot et al., 2000; Jensen et al., 2005). In general, leaves have higher rates of C and N mineralization than stems (Cobo et al., 2002; Abiven et al., 2005; Thippayarugs et al., 2008) due to their more readily decomposable tissue composition, lower lignin content and higher total N content (Quemada and Cabrera, 1995; Bertrand et al., 2006). A low availability of soil mineral N can slow the decomposition of crop residues with high C:N ratios due to a lack of assimilable N available to soil decomposers (Recous et al., 1995). This N limitation may also be an indirect effect of the limited contact between soil and plant residues at the soil surface (Coppens et al., 2007).

Many studies have been performed to better understand the effect of plant residue quality on C or N mineralization using plant leaves, stems or mixtures (shoots), with residues incorporated into the soil (e.g., Bertrand et al., 2006; Abiven et al., 2005; Thippayarugs et al., 2008) or deposited on the soil surface (Quemada and Cabrera, 1995; Cobo et al., 2002, Li et al., 2013). However, in field conditions, different types of plant residues often decompose together in a mixture (Hättenschwiler et al., 2005). The C and N mineralization of litter mixtures can differ from that expected based on the decomposition of single components because the composition of the residue can modify the processes involved in decomposition (Hoorens et al., 2002; Gartner and Cardon, 2004; Berglund et al., 2013). The decomposition of residue mixtures (or their C and N mineralization) has been reported to exhibit synergistic effects (i.e., higher rates of

decomposition than expected) (Quemada and Cabrera, 1995; Zeng et al., 2010), negative effects (i.e., lower rates than expected) or additive effects (i.e., rates equal to those expected) (Liu et al., 2007; Li et al., 2013a; Li et al., 2013b). However, high variations in the range of responses of decomposition rates to mixing have been observed (Gartner and Gardon, 2004) depending on the type of residue, time scale and process considered (e.g., mass loss, C mineralization or N dynamics). Non-additive effects of mixing residues are assumed to result from the chemical heterogeneity of the mixtures (Harguindeguy et al., 2008) and particularly to the transfer of N between N-rich and N-poor litters (Berglund et al., 2013). Indeed, this question is attracting much attention, particularly in the field of ecology, in investigations of the role of functional diversity (in this case, diversity of plant traits) on ecosystem functioning (Hättenschwiler et al., 2005). Trait similarity measures are used to characterize differences in chemical and physical characteristics of plant litters and to analyze non-additive effects (Gessner et al., 2010; De Bello et al., 2013). These approaches have been little or not at all under agricultural conditions (Garnier and Navas, 2012).

Thus, the primary objective of the present study was to investigate, in an agricultural context, the effect of mixing crop residues (leaf and stem) at the soil surface on their decomposition (C mineralization) and the associated soil N dynamics. We used residues (leaves, stems and mixtures of leaves and stems) with a wide range of chemical characteristics obtained from 25 different crop species from five botanical families, mainly Fabaceae and Poaceae, and incubated them under controlled conditions. We hypothesized that the response to mixing would be highly dependent on the respective chemical composition of leaves and stems and their proportion in the mixtures and that these factors would affect C and N dynamics differently.

4.3 Materials and methods

4.3.1 Collection of plant material

Twenty-five representative species of plants (main crops or cover crops) of agricultural systems in Brazil were studied (Table 1). The plants selected included eleven Fabaceae (legumes), ten Poaceae (Gramineae), two Brassicaceae, one

Euphorbiaceae and one Asteraceae. The plants were cultivated during the spring/summer (14 species) and autumn/winter (11 species) in a Typic Hapludalf soil under a no-till system in the experimental area of the Soil Department (29°41′ S, 53°48′ W; approximately 90 m elevation) of the Federal University of Santa Maria in the state of Rio Grande do Sul, Brazil. The region has a subtropical climate with a mean annual precipitation of 1,686 mm and a mean air temperature of 19.3°C. For the previous 12 years, the experimental site had been cultivated using a no-till system. All the crops were managed according to the technical recommendations for the area. Plant shoots with 3 field replicates were collected at flowering and harvest for species of cover crops and main crops, respectively. The leaves senescing before harvest were collected gradually until harvest and stored. Subsequently, the plant shoots were separated into leaves and stems to determine their proportion in each plant species (Table 1). First, the residues were dried at 40°C and the leaves and stems were cut into pieces 1 cm long. Then, the residues were cut again along the length into pieces with a thickness of approximately 0.5 and 0.3 cm for leaves and stems, respectively. Mixtures of leaves and stems were prepared with a leaf:stem ratio for each species similar to the ratio in dry biomass determined in the field (Table 1). One subsample of each residue (leaf, stem and mixture) per species dried at 40°C was ground to 1 mm particles, and a second subsample of each type of residue was dried at 65°C and finely ground (<1 mm) for chemical analyses.

4.3.2 Chemical characterization of plant residues

The total organic C and total N contents of the leaves, stems and mixtures of leaves + stems were determined in a finely ground subsample dried at 65°C using an elemental autoanalyzer (FlashEA 1112, Thermo Finnigan, Milan, Italy). A proximate analysis using the Van Soest method was performed using subsamples of ground residues pre-dried at 40°C. The soluble (SOL), cellulose (CEL), hemicellulose (HEM) and lignin (LIG) fractions of the residues were determined by proximate analysis (Van Soest, 1963). Neutral digestion was performed with 0.3 g of residues and 30 ml of neutral detergent solution. For acid digestion, 0.6 g of residues and 60 ml of detergent solution acid were used. The digestions were performed by boiling the material in the digester block at 150°C for 1 hour. After digestion, the samples were filtered by vacuum

suction using a filter crucible and then washed with hot distilled water (90°C) and acetone (30-40 ml). The fibers were dried at 105°C for 12 hours. The SOL content was determined based on the difference in the weight before and after neutral digestion. The HEM content was determined based on the difference between the percentage of total neutral fiber and the percentage of acid detergent fiber. The CEL content of the acid fiber was obtained after digestion with 12M H_2SO_4 for 3 hours, and the material remaining after quantification of the CEL content was burned in a muffle furnace at 500°C for 3 hours to determine the LIG content.

The water-soluble organic C (Csw) and water-soluble total N (Nsw) were extracted using 0.5 g of finely ground residues pre-dried at 40°C. The residues were placed in a 60-ml snap cap with distilled water (20°C) and mechanically stirred for 30 minutes. After mixing, the material was filtered (Whatman n°5), and the contents of Csw and Nsw in the filtrate were determined at 65°C. The quantification of soluble polyphenols (POL) was conducted with 0.75 g of finely ground residues pre-dried at 40°C. POL extraction was performed with aqueous methanol (1:1) in a water bath at 80°C for 1 hour (Tian et al., 1995). After extraction, the aqueous solution was filtered (Whatman n°2). After addition of the Follin-Denis reagent, the POL content was determined by colorimetry (absorbance at 760 nm) (King and Health, 1967). All the analyses were performed with three replicates, and the summarized results are shown in Table 2.

4.3.3 Soil, treatments and experimental conditions

The soil used in the incubation experiment was a Typic Hapludalf (USDA classification) collected from the 0-10-cm layer in the same area as the plants. This soil contained 120 g kg^{-1} clay, 280 g kg^{-1} silt, 600 g kg^{-1} sand, 8.7 g kg^{-1} organic C and 0.9 g kg^{-1} total N, with a pH (soil H_2O) of 5.4. The soil initially contained 9.4 mg mineral N kg^{-1} dry soil. After visible organic residues had been removed by hand, the soil was sieved to 4 mm. Distilled water was applied to the soil to achieve a soil moisture content of 80% of field capacity. The soil was pre-incubated in plastic bags at 25°C for 5 days. The experiment consisted of incubations conducted for 120 days in the dark at 25±1°C to measure the C and N mineralization of leaves, stems and mixtures of leaves + stems. The experimental design consisted of two sets of jars prepared and monitored in

parallel, with one jar used to evaluate C dynamics and one to evaluate N dynamics. The 76 treatments consisted of a non-amended soil (as a control), soil + leaves, soil + stems and soil + mixture (leaves + stems) for the 25 plant species listed in Table 1 with three replicates per treatment and per sampling time. The treatments were arranged in a completely randomized design, and each treatment was replicated three times. For each replicate, a sample of 134 g of soil at 13.8% moisture (equivalent to 80% of field capacity) was used. First, a moist soil subsample (67 g) was placed in a 110-ml cylindrical acrylic pot (5.0 cm in diameter and 5.0 cm in height) and compressed to a height of 2.5 cm. A second soil subsample (67 g) was placed in the same acrylic pot and compressed to a total height of 5 cm. Thus, the soil in each pot reached a final bulk density of 1.2 g cm^{-3}. The residues were applied on the surface of the soil at a rate of 0.56 g DM/pot (equivalent to a basis of 4.74 g DM per kg of dry soil or a rate of 3.06 Mg DM ha^{-1}), which was equivalent to the addition of 1790 (rice stem) to 2210 (dwarf pigeonpea leaf) mg C kg^{-1} of dry soil and 13 (sunflower stem) to 220 (gray mucuna leaf) mg N kg^{-1} of dry soil. Each acrylic pot was placed in a 1,000-ml glass jar prior to incubation.

4.3.4 Analytical procedures

The C mineralization of the crop residue mixtures was assessed by quantifying the CO_2 release. The CO_2 produced in the soil was trapped in 10 ml of 1 M NaOH in a beaker placed inside each glass jar. The NaOH beakers were changed at each sampling, i.e., at 2, 4, 7, 10, 14, 21, 28, 35, 50, 70, 90 and 120 days. The carbonate trapped in the NaOH was precipitated with a 2 M $BaCl_2$ solution, and the remaining NaOH was back-titrated with 1 M HCl. At all sampling times, the jars were aerated for 10 minutes to renew the internal atmosphere and the soil water content was checked by weighing and adjusted as necessary. The apparent C mineralization of the crop residue mixtures was calculated by subtracting the amounts of CO_2-C evolved from the control treatment from the amounts of CO_2-C evolved from the amended treatments. Apparent mineralization assumes that there is no effect of crop residue addition on soil C mineralization (no priming effect) or that this effect is similar regardless of the type of crop residue added.

The soil mineral N ($NH_4^+ + NO_2^- + NO_3^-$) was measured in all treatments at time 0 and at 7, 14, 21, 35, 63, 90 and 120 days of incubation. At each sampling time, the visible residue particles were removed from the soil surface. The mineral N was extracted from fresh soil samples with 1 M KCl (30 minutes shaking and a soil:solution ratio of 1:4. The soil KCl suspension was settled for 30 minutes until the supernatant liquid was clear, and the mineral N in a soil extract aliquot was measured by steam distillation (Keeney and Nelson, 1982). The jars were opened periodically and aerated and the humidity was adjusted when necessary. The net N mineralization was calculated by subtracting mineral N in the control from the amount of mineral N accumulated in each amended treatment.

4.3.5 Data and statistical analyses

Data of N mineralization and of cumulative mineralized C measured over 120 days were analyzed by one-way analysis of variance. Least significant difference tests ($P<0.05$) were used to determine whether significant differences occurred.

To obtain a quantitative measure of the relative importance of the initial chemical characteristics of residues in determining residue mineralization, we first calculated C mineralization using an exponential equation according to Jung et al. (2011) as follows:

$$Cmin = C_0 (1 - e^{-k\,\mathrm{day}}) \qquad (1)$$

where *Cmin* is the amount of mineralized C, C_0 is the potentially mineralizable C pool, k is a mineralization constant and day is the incubation period. Stepwise multiple regression analysis was then used to determine which combinations of chemical variables best explained the variations in C_0 and k. Only those variables that were found to be significant at $P<0.05$ were retained in the regressions. Regressions were performed with all available chemical variables of residues.

A principal component analysis (PCA) was also performed to investigate the chemical traits that differentiate plant organs and plant families according to Aulen et al. (2012). PCA was performed on the correlation matrix obtained from the proximate analysis (i.e., SOL, CEL, HEM and LIG), C, N, water-soluble fractions (i.e., Csw and

Nsw) and POL. The potentially mineralizable C pool (C_0) and the mineralization constant (k) were added as supplementary variables (i.e., not included in the ordination of species) to analyze their relationships with chemical characteristics. The PCA produced an ordination of the species and the chemical composition of the leaves, stems and mixtures, which were plotted in one and two dimensions, respectively, based on the scores of the first two principal components (PCs). A correlation circle was computed to assess the importance of each chemical component in the PC axes. We considered r values (i.e., the correlation coefficient) as significant contributors when $r \geq 0.5$ (Guénon et al. 2013).

To address the question of the non-additivity of mineralization in residue mixtures, we compared the evolved CO_2, soil mineral N content and net N mineralization in residue mixture treatments ("observed") to the "expected" values for each crop species. The expected C-CO_2 mineralized from crop residue mixtures at days 14 and 120 was calculated as the weighted mean of the evolved leaf-derived C-CO_2 and stem-derived C-CO_2 according to their respective proportions in the mixture (Table 1). This procedure assumes an additive effect of mixing the two types of residues. A similar calculation was performed for the net mineralization of N. The calculated values were compared to the observed values, and differences were analyzed using Tukey's test. Based on the results of the statistical analysis, it was possible to classify residue interactions for C and N mineralization into 3 types as follows (Robinson et al., 1999): (1) measured and expected values do not significantly differ, and the contribution of each residue type to the mixture is additive; (2) measured values are significantly greater than expected values, indicating that mixing the residues has a positive (or synergistic) effect; and (3) measured values are significantly lower than expected values, indicating that mixing the residues has a negative effect (or antagonistic) on mineralization.

Deviation from additivity (Da) was calculated using the signed non-additivity calculation according to Makkonen et al. (2013) as follows:

$$\mathrm{Da} = \left(\frac{\text{observed CO2 (or Nmin)}}{\text{expected CO2 (or Nmin)}} \right) - 1 \qquad (2)$$

We used a multiple-trait-similarity measure to characterize differences in the chemical traits (composition) of the leaf and stem residues of each mixture for each

species (De Bello et al., 2013) and to assess the effect of similarity in functional traits on non-additive effects. According to Gower and Legendre (1986) and Pavoine et al. (2009), we used Gower's (1971) general coefficient of similarity applied to quantitative variables with only positive and no missing values. The similarity between leaf residue *i* and stem residue *j* for a variable *k* is measured as the difference between different traits, and this difference is standardized between 0 and 1 for each trait as follows:

$$S_{ijk} = 1 - |X_{ik} - X_{jk}| / R_k \qquad (3)$$

where S_{ijk} is the similarity between residue *i* and residue *j* calculated for the *k* the variable; X_k is the measured variable; and X_i and X_j are the values taken by the X_k variable of residues *i* and *j*, respectively; and R_k is the range of X_k variables measured. In this work, R_k was taken for each variable as the maximal difference between all values of the variable in the entire population (25 species) as follows:

$$R_k = \text{Max}\ (X_{ik} - X_{jk}) \qquad (4)$$

As the leaf:stem ratio in the mixtures were not 50:50 but, rather, depended on the leaf:stem ratios in the field, S_{ijk} was weighted according to the proportion of the two plant parts in the mixture. The values of S_{ijk} and S_{ij} lie in the interval of 0 to 1. In the present study, the so-called "species" of the original method were leaf and stem, and the data set included 25 pairs of leaves and stems (corresponding to the 25 plant species studied) and 9 variables corresponding to the chemical characteristics (or traits) measured. The relationships between the average additivity value and the single-trait similarity (S_{ijk}) were analyzed by linear regression. The traits that showed significant regressions with additivity for C or for N were selected to build the multiple trait similarity coefficients (S_{ij}).

4.4 Results

4.4.1 Chemical quality of leaves, stems and mixtures

The parameters of the chemical composition (minimum, maximum and mean values) of leaves, stems and mixtures (leaf + stem) of the 25 plant species are summarized in Table 2. The residue C and N contents ranged from 387 to 486 g kg^{-1} and from 2.6 to 51.8 g kg^{-1}, respectively, resulting in a C:N ratio ranging from 8.6 to 178. The measured chemical compositions of the residue mixtures were close ($r^2 \geq 0.96$) to the expected chemical composition (data not shown). The chemical characteristics were analyzed by a PCA to extract the most significant differences between organs and species (Fig. 1). The PCA showed that the first two PCs accounted for 70% of the total variance of the data. PC 1 explained 55% of the variance of the data and separated leaves of Fabaceae and Poaceae species as follows: in the positive part of the PCA, leaves from Poaceae species were most strongly correlated with CEL (r=0.91) and HEM (r=0.76) contents; and in the negative part of the PCA, leaves from Fabaceae species were most strongly correlated with SOL, total N, POL and water-soluble fractions (in the range of r=-0.65 to -0.97) (Fig. 1a, c). PC 2 explained 15% of the variance of the data and separated stems of Poaceae from those of other families (Fabaceae, Asteraceae, Brassicaceae and Euphorbiaceae) as follows: in the negative part of the PCA, stems from Poaceae species were slightly correlated with HEM content (r=-0.32) and stems from other families were correlated with LIG content (r=0.78) (Fig. 1a, b, c). The mixture (leaf + stem) (Fig. 1a) showed an intermediate distribution between leaves and stems for PC 1 and PC 2, as expected. When analyzed only according to botanical families (Fig. 1b, c), PC 1 clearly showed that all residues from Poaceae species (stems, leaves and mixtures) were close to each other and were strongly correlated with CEL (r=0.91) and HEM (r=0.76) contents. Conversely, the distribution of residues from Fabaceae species was much wider along the two axes.

Similarity coefficients for each individual chemical characteristic and aggregated similarity coefficients expressed the extent of differences among the chemical traits of leaves and stems for each species. Varied conditions occurred in this study. For example, sunn hemp and oilseed radish exhibited the widest variation in chemical composition between leaves and stems (S_{ij}=0.28), and rice exhibited a similar composition in leaves and stems for all criteria (S_{ij}=0.93) (Supplementary data A). As expected from the PCA, the similarity coefficients also effectively expressed the differences between families, with S_{ij}=0.46±0.12 for Fabaceae and S_{ij}=0.81±0.20 for Poaceae. Other families (represented by one or two species; i.e., Brassicaceae, Euphorbiaceae and Asteraceae) had a low S_{ij} value, indicating a large difference in the

chemical traits between stems and leaves. The "weighted" similarity coefficients reflected the actual contribution of leaves and stems to chemical traits by taking into account their actual proportion in mixtures. The range of (weighted) S_{ij} values was narrowed and varied from 0.44 (pea) to 0.96 (rice) (Supplementary data B). The average weighted S_{ij} changed to 0.60±0.09 for Fabaceae species, but it remained unchanged for Poaceae species (0.81±0.16).

4.4.2 C and N mineralization

The cumulative C and N mineralization in soils amended with leaves, stems and mixtures varied widely among species (Fig. 2). The cumulative apparent C mineralization at day 120 ranged from 44±1.0 to 67±2.2% added C for leaves (Fig. 2a), 26±2.8 to 62±2.7% added C for stems (Fig. 2c) and 39±1.8 to 63±1.7% added C for the mixtures (Fig. 2e). The net N mineralization in soil showed a contrasting pattern among residue types in different species. Most of the leaf treatments induced a net increase in soil mineral N (+141 to -20 mg N kg^{-1} soil at day 120) (Fig. 2b), but stem decomposition induced stronger immobilization, with net mineralization in the range of +53 to -38 mg N kg^{-1} soil at day 120 (Fig. 2d). As expected, the mixtures generated intermediate results (+79 to -26 mg N kg^{-1} soil) (Fig. 2f, Table 2). In several conditions, the mineral N content in soil was almost depleted during the first 14 days and remained depleted until of the end of incubation (120 days), as observed for both residues of maize (3), rye (15) and barley (19) species (Fig. 2b, d, f). The net N mineralization was positively and linearly related to the residue N concentrations, with residue with low N content (high C:N ratio) inducing a net immobilization (Fig. 3).

The C_0 of residues represented, on average, 46.1% added C for stems and 56.0% added C for leaves, and the values were close to the observed cumulative C mineralized at day 120 (Table 2). As expected, the mineralization constant (k) of the C_0 differed among residues and ranged from 0.012 (sunflower stem) to 0.128 (oilseed radish leaf) (Table 2; Supplementary data C). The PCA analyses indicated that total N, total soluble polyphenols and soluble fractions (Van Soest; water-soluble N and C) were correlated with a high C_0 and with k when considering all residues (stems, leaves and mixtures) (Fig. 1c). As analyzed by stepwise regression with standardized coefficients, the relationships between k or C_0 and the initial residue chemical composition varied

greatly according to plant organ (leaf vs. stem vs. mixture) and to the range of species considered (Table 3). N content was the greatest explanatory chemical trait for leaves (r^2≥0.78), which was not the case for stems, for which HEM and/or CEL were more significant (r^2≥0.42). For mixtures, the hierarchy of traits was close to that of stems for k (r^2=0.78) and to that of leaves for C_0 (r^2=0.42). Within each family (Fabaceae or Poaceae), other chemical traits were selected. The LIG content was never selected, except for k in mixtures and Poaceae stems (r^2≥0.66). The k and C_0 in mixtures of Poaceae species were strongly explained by total N content (r^2≥0.82). All of the equations were highly significant (P<0.001).

4.4.3 Interactions between leaf and stem in mixtures

The relationship between observed and expected mineralization was analyzed using residue C mineralization and soil N content at days 14 (early decomposition) and 120 (end of incubation) in addition to the mineralization constant k. Most of the statistically significant differences (conditions of non-additivity) observed at day 120 had been previously obtained at day 14 for C and N (Supplementary data D), but the results discussed here concerning the entire incubation period are values obtained after 120 days of incubation. Overall, the effect of mixing residues was low for C mineralization (Fig. 4a; Supplementary data D) with the following effects: additive for 15 mixtures, positive for 7 mixtures and negative for 3 mixtures. The positive or synergistic effect (mean +9% added C mineralized) occurred with mixtures of sunflower (4), vetch (12), wheat (13), oilseed radish (14), oilseed rape (18), ryegrass (20) and dwarf pigeonpea (22). The negative or antagonistic effect (mean -5% added C mineralized) occurred with mixtures of bean (1), showy rattlebox (10) and rye (15).

The effects of mixing on soil mineral N content differed from the effect on C mineralization (Fig. 4b; Supplementary data D) with the following effects: additive for 18 species; positive for 2 mixtures, i.e., showy rattlebox (10) and castor bean (21); and negative for 5 mixtures, i.e., jack bean (6), gray mucuna (7), vetch (12), pea (16) and dwarf pigeonpea (22). All Poaceae mixtures showed additive effects for mineralized N in soil. For most of the species, a non-additive effect of mixing on C mineralization was not associated with a non-additive effect on N mineralization. Overall, there were no clear relationships between mixture effects on the C or N cumulative mineralization and

the degradation constant *k*. The 7 mixtures showing positive effects on C mineralization (Fig. 4a) tended to have additive or negative effects on the degradation constant *k* (Fig. 4c), particularly for the sunflower (4) and ryegrass (20) mixtures. The bean (1) and showy rattlebox (10), which had slight negative effects on C mineralization, also showed slight effects on *k* (additive). Sunn hemp (5), which had a stronger effect on the *k* constant, did not have any significant effect on the amount of C or N mineralization, suggesting that the kinetics of mineralization for this species may have been modified by mixing without any significant difference between the observed and expected amount of C or N mineralized at day 120.

To further analyze the underlying pattern of responses among the litter mixtures, we analyzed the global response using all mixtures. There was a significant linear relationship between observed and expected C mineralization for the 25 mixtures (r^2=0.83; P<0.05) (Fig. 5a). Mixtures with a cumulative C mineralization > ~1100 mg C-CO_2 kg^{-1} soil (i.e., higher mineralized C at day 120) exhibited a ratio close to 1 (observed:expected). These mixtures were mainly of Fabaceae species. For most of the mixtures with cumulative mineralization < ~1100 mg C-CO_2 kg^{-1} (lower mineralized C), the observed C mineralization differed from the expected mineralization and was either higher or lower depending on the species. These mixtures were primarily of Poaceae species. Regarding soil N, there was also a significant linear relationship between observed and expected net N mineralization at day 120 (r^2=0.94; P<0.05) (Fig. 5b). On the positive side of the N mineralization axis, mixtures were mainly from Fabaceae species, and the relationship indicated that the observed N mineralization was slightly lower than expected. On the negative side of the N mineralization axis, mixtures were mainly from the Poaceae species, and the relationship indicated that there was less N immobilized than expected. The results were confirmed when N mineralization values (observed or expected) were normalized by the differences in C mineralization (observed or expected), i.e., expressed in N mineralized per g of C mineralized for each mixture, to take into account possible differences in decomposition (Fig. 5c).

The effect of similarity in functional traits on non-additive effects between the two components in a given residue litter was analyzed by linear regression. Single-trait similarity (weighted S_{ijk}) was first regressed against non-additivity. Traits associated with non-additive effects were then used in combination (multiple-trait similarity). Non-additive effects in litter mixtures for C mineralization and degradation constant (*k*) were related neither to any single-trait similarity coefficient nor to the full aggregated

similarity coefficient (data not shown). For N mineralization, the Da for the 25 mixtures was slightly but significantly related to total N, water-soluble N, Van Soest SOL and water-soluble C (data not shown). The best relationship was obtained using only soluble N (r^2=0.25; P<0.01). Da increased (from negative values to positive values) with increasing weighted S_{ijk} for total N, indicating that synergistic effects on N mineralization occurred with decreasing heterogeneity in the N content of the mixtures (r^2=0.24; P<0.01). Using only the 7 species that had a significant non-additive effect on net N mineralization (6 Fabaceae and one Euphorbiaceae), we observed that the Da was negatively related with the N content of the mixtures (Fig. 6a) and positively related to S_{ij} for total N (Fig. 6b). The Fabaceae species, which exhibited high N content and high similarity between leaf and stem, also exhibited high SOL content (Fig. 1).

4.5 Discussion

The diversity of the initial quality was created by using mixtures of leaves and stems from 25 different species, primarily including Fabaceae (legumes) and Poaceae (Gramineae). These species were characterized by their botanical family membership and by their maturity as follows: residues harvested at plant maturity (main crop) and residues harvested in the vegetative phase (cover crops). The mixtures resulted in different chemical compositions both between species for the same organ (leaf or stem) and between leaves and stems for a given species. For example, most Fabaceae species were collected in the vegetative phase and were characterized by high levels of N and soluble compounds. Conversely, most grass species were harvested at maturity, and their tissues were richer in cellulose, hemicellulose and lignin, as expected (Bertrand et al., 2009). These results were striking due to the diversity of the compositions, which showed a relatively low diversity among species and between leaves and stems for Poaceae species but greater diversity for Fabaceae. The quality of the mixtures used in the present study also included a second factor of heterogeneity, which was the proportion of stems and leaves in the mixture. The proportion studied was based on measurements taken in the field, with the objective of mimicking the actual agricultural conditions. Mixtures of leaves and stems exhibited a narrower range of chemical compositions than residues composed of stems or leaves alone, as expected, but in some cases also to a reversing of the hierarchy species. Such was the case, for example, with

the jack bean, pea and vetch mixtures that included a large proportion of leaves. The chemical quality of mixtures can therefore be characterized by the average chemical composition and by the degree of chemical heterogeneity of the mixture, as has been discussed by Harguindeguy et al. (2008).

We used the Gower coefficient of similarity proposed by Gower and Legendre (1986) and De Bello et al. (2013) to quantify this heterogeneity. The results confirmed a marked typology based on the botanical family to which the species belonged (S_{ij}=0.4 for Fabaceae and S_{ij}=0.8 for Poaceae mixtures), and the results identified species for which both organs were similar in all measured criteria (e.g., S_{ij}=0.93 for rice) or different (e.g., S_{ij}=0.28 for oilseed radish). These results showed that the effect of the mixtures must be studied according to two characterizations: the average composition of the mixture and the heterogeneity of the mixture. Given the characterizations, we assumed that mixtures with low heterogeneity (e.g., Poaceae) would likely undergo little or no effect as a result of the mixing of leaves and stems. However, this study was limited to the examination of chemical features and did not account for differences in physical properties, which can change the decomposition of residues left on the surface of the soil. This is the case, for example, for the capacity of water retention by plant residues (Iqbal et al., 2013) or the micro environmental conditions (Makkonen et al., 2013), which can alter the moisture of mulches depending on the climate and influence the decomposition within a mixture. Under the conditions of decomposition at the soil surface, as in this experiment, it can be assumed that the low availability of N in the low N residues will be limiting.

The C mineralization was strongly dependent on the initial chemical composition, which confirmed the findings of numerous studies on annual crops (Trinsoutrot et al., 2000; Jensen et al., 2005) and perennial crops from natural ecosystems, for which the relationship between functional traits and decomposition have been extensively studied (e.g., Berglund et al., 2012). We noted a strong correlation between the chemical constituents and mineralization of C (C_0 and k) for all leaves, with significant differences according to the N content when the 25 types of leaves were considered together. Within the two main families, the determinants of mineralization differed. Most of the leaves underwent immediate decomposition (mainly in Fabaceae species), which was related to the relatively high SOL, total N, POL and water soluble fractions in leaves. The observed higher decomposition was in agreement with other studies (Cobo et al., 2002; Abiven et al., 2005; Thippayarugs et

al., 2008), confirming the importance of soluble fractions (mainly in early stages) in plant residue decomposition (Reinertsen et al., 1984; Trinsoutrot et al., 2000; Jensen et al., 2005). In contrast, leaves and stems of the Poaceae family exhibited gradual decomposition, resulting in more gradual and stronger immobilization than exhibited by Fabaceae species. These results for leaves and stems can be attributed to the presence of higher cellulose and hemicellulose contents but also to low N content (total and soluble) combined with limited N availability in the soil. The lignin content did not appear to regulate the residue decomposition rates in soil. Using 47 different crop residues, Trinsoutrot et al. (2000) also found that lignin does not explain the mineralization constant *k*. Using 249 different above-ground residues from crops, Jensen et al. (2005) found that holocellulose (CEL + HEM) is the factor that explains the greatest variability of C mineralization. The soluble polyphenol content was highly correlated with the soluble pool in Fabaceae residues (r^2=0.72; P<0.001). Thippayarugs et al. (2008) also showed that the decomposition of leaves of Fabaceae species is correlated with a higher total N than that found in the decomposition of stem residues.

The amount of mineral N in the soil under the residue layer changed dramatically over the course of the decomposition process and greatly depended on the nature of the residues, indicating that exchanges between the residue layer and the underlying soil were effective both in terms of diffusion of C and/or N from the residues to the soil, which provoked soil N immobilization or N accumulation, and diffusion of N to the residue layer to feed N decomposers. Only isotopic approaches can quantify the importance of the different physical- and biological-mediated exchanges between soil and residues (Lummer et al., 2012; Berglund et al., 2013). The total N in tissue residues played an important role in the processes of decomposition and N mineralization, particularly with residues at the soil surface and under conditions of low soil mineral N availability. This result can be correlated with the positive relationship between total N residues and soil N net mineralization. The fact low N mineral content of the soil at the beginning of incubation in our study (approximately 9 mg kg^{-1} soil) likely contributed to retardation in the colonization of plant tissues by microorganisms, a slower C mineralization rate and reduced N immobilization (Recous et al., 1995). In the present study, the pattern of responses was similar to results obtained with incorporated residues (residues with low N content, mainly stem and grass mixtures), exhibiting a decrease of the net mineral N in the soil. Residues with a higher N content (mostly leaves, stems and mixtures of cover crops) resulted in a net increase in the amount of mineral N in the

soil. The kinetics of soil N evolution differed depending of the residues, with some temporary net immobilization followed by net mineralization. The mixtures exhibited a narrower range of N contents, but the relationship remained the same.

The effect of mixing different types of residues (leaf + stem) was quantified in two ways: by calculating the Da for each mixture, as proposed by Makkonen et al. (2013), and by establishing regressions between observed and expected data for mineralized C and net soil mineralized N at day 120. The dynamics of non-additivity were also measured over time (particularly at day 14), but the results did not change the conclusions and are not detailed. The two ways of calculating this effect provided slightly different results. This study showed that the effects of mixing residue types on C and N mineralization were different. Therefore, it is important to distinguish between C and N mineralization when analyzing interactions in residue mixtures. In fact, the literature shows a synergistic effect of mixed residues on the degradation of C, which is often measured by mass loss (Gardner and Cardon, 2004). However, our results showed a majority of additive responses (no interactions), and only approximately one-third of these responses were positive. However, the overall positive effect was low and variable, and it was often not significant, which confirmed the findings of most previous studies (Harguindeguy et al., 2008; Makkonen et al., 2013). This result was confirmed by establishing the relationship between expected and observed mineralized C for each of the 25 species. In this case, we found that positive or negative, but not always statistically significant, effects were obtained for mixtures lower mineralization rates throughout the duration of the experiment, i.e., predominantly Poaceae. N is potentially a limiting factor for optimal decomposition at the soil surface and it may have been responsible for this effect, either increasing or decreasing the mineralization rate of mixtures. For C mineralization, none of the indicators (including tissue N) were significantly related to the response (additive or non-additive), which may have been due to the weak effect between residue type and decomposition rate. This result may seem surprising but in fact corresponds to the findings of other authors (e.g., Makkonen et al., 2013).

Regarding N, the Da calculated by species showed that the non-additivity was limited to a small number of residues and was mostly negative, which indicated that the observed net mineralization of N in soil under the residue layer was lower than expected. This trend applied only to a few Fabaceae mixtures with high N content and a greater heterogeneity of the mixture for N content, which supports the idea of a main

interaction effect of nutrient contents, especially N, as has been shown in several studies (Hättenschwiler et al., 2005; Berglund et al., 2012). Berglund et al. (2012) used differences in ^{13}C natural abundance between ^{15}N-labeled *Pinus* and maize litters to quantify the bidirectional transfer of C and N between litters. The fact that the negative deviation for N was not accompanied by a positive or negative deviation for C suggests that the lower net accumulation of N in the soil did not correspond to a reduced C decomposition or to an increased decomposition of the mixture, which would have caused an increased N immobilization. Our hypothesis is that N, which accumulated in smaller quantities than expected in soils with mixtures of pea, jack bean, vetch, gray mucuna and dwarf pigeonpea, was immobilized in greater quantities by decomposer organisms growing on the residues themselves. An analysis of chemical characteristics at the end of experiments and the use of isotope tracing for N and C could help answer this question. The analysis of Da performed on the 25 mixtures (regression between expected N mineralization vs. mineralization observed at 120 days) confirmed the effect of mixed residues on N mineralization, with a Da (positive or negative but not always significant) under conditions of high mineralization observed with mixtures of Fabaceae. The main explanatory factors were the N content of the mixtures and their degree of heterogeneity for this chemical trait.

4.6 Conclusions

This study showed that mixtures of plant residues consisting of leaves and stems of 25 species of annual crops (main crops and cover crops) induced a broad range of chemical compositions and chemical heterogeneity of the mixtures. With regard to the effects of the functional traits of plants (and plant litters) on important functions of agro-ecosystems, such as mineralization, the results indicated that the synergistic, antagonistic or simply additive effects of mixtures varied, depending on the process considered (i.e., in this study, the mineralization of C or N). In our experimental conditions, these effects were relatively low on average and were not always significant and varied depending on the mixture considered. The occurrence of non-additive or additive effects depended on the range of the chemical quality of the mixtures (e.g., the Da for N in the low C:N range of mixtures represented by Fabaceae cover crops), and the intensity of effects (positive or negative) was primarily linked to the heterogeneity

of the mixture quality, i.e., the synergistic or antagonistic effects between the components of the mixture. In all cases, the results suggested that the availability of mineral N involved in the interactions between the mixture components and in the interactions between the residues and the underlying soil played an important role in the occurrence and sign of the non-additivity, confirming the results of most recent works. The characterization of mixtures according to botanical family and plant organ is appropriate for studying the overall composition of plant parts considered individually or in combination and their effects on mineralization. Our results show that an approach involving the characterization of functional traits based on the chemical attributes of litter types and using the Gower coefficient of similarity commonly applied in research on natural ecosystems can be effectively applied to agro-ecosystems.

Acknowledgments

This work was supported by the Brazilian government through the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). The bilateral Brazilian and French collaboration was funded under the Program CNPq - Ciência sem Fronteiras (Process Number 401724/2012-3) - and by the INRA (the Environment & Agronomy Division) during M. Redin's residence at UMR FARE in Reims, France. We also thank Dr. Nicolas Fanin for constructive discussions on the topic of mixtures.

4.7 References

Abiven, S., Recous, S., Reyes, V., Oliver, R., 2005. Mineralisation of C and N from root, stem and leaf residues in soil and role of their biochemical quality. Biology and Fertility of Soils 42, 119-128.

Aulen, M., Shipley, B., Bradley, R., 2012. Prediction of in situ root decomposition rates in an interspecific context from chemical and morphological traits. Annals of Botany 109, 287-297.

Berglund, S.L., Agren, G.I., Ekblad, A., 2013. Carbon and nitrogen transfer in leaf litter mixtures. Soil Biology & Biochemistry 57, 341-348.

Berglund, S.L., Agren, G.I., 2012. When will litter mixtures decompose faster or slower than individual litters? A model for two litters. Oikos 121, 1112-1120.

Bertrand, I., Chabbert, B., Kurek, B., Recous, S., 2006. Can the biochemical features and histology of wheat residues explain their decomposition in soil? Plant and Soil 281, 291-307.

Bertrand, I., Prevot, M., Chabbert, B., 2009. Soil decomposition of wheat internodes of different maturity stages: Relative impact of the soluble and structural fractions. Bioresource Technology 100, 155-163.

Cobo, J.G., Barrios, E., Kass, D.C.L., Thomas, R.J., 2002. Decomposition and nutrient release by green manures in tropical hillside agroecosystem. Plant and Soil 240, 331-342.

Coppens, F., Garnier, P., Findeling, A., Merckxb, R., Recous, S., 2007. Decomposition of mulched versus incorporated crop residues: Modelling with PASTIS clarifies interactions between residue quality and location. Soil Biology & Biochemistry 39, 2339-2350.

De Bello, F., Vandewalle, M., Reitalu, T., Leps, J., Prentice, H.C., Lavorel, S., Sykes, M.T., 2013. Evidence for scale-and disturbance-dependent trait assembly patterns in dry semi-natural grasslands. Journal of Ecology 101, 1237-1244.

Garnier, E., Navas, M-L., 2012. A trait-based approach to comparative functional plant ecology: concepts, methods and applications for agroecology. A review. Agronomy for Sustainable Development 32, 365-399.

Gartner, T.B., Cardon, Z.G., 2004. Decomposition dynamics in mixed-species leaf litter. Oikos 104, 230-246.

Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H., Hättenschwiler, S., 2010. Diversity meets decomposition. Trends in Ecology and Evolution 25, 372-380.

Gower, J.C., 1971. A general coefficient of similarity and some of its properties. Biometrics, 27, 857-874.

Gower, J., Legendre, P., 1986. Metric and Euclidean properties of dissimilarity coefficients. Journal of Classification 3, 5-48.

Guénon, R., Vennetier, M., Pailler, A., Dupuy, N., Roussos, S., Gros, R., 2013. Trends in recovery of Mediterranean soil chemical properties and microbial activities after infrequent and frequent wildfires. Land Degradation and Development 24, 115-128.

Harguindeguy, N.P., Blundo, C.M., Gurvich, D.E., Diaz, S., Cuevas, E., 2008. More than the sum of its parts, Assessing litter heterogeneity effects on the

decomposition of litter mixtures through leaf chemistry. Plant and Soil 303, 151-159.

Hättenschwiler, S., Tiunov, A.V., Scheu, S., 2005. Biodiversity and litter decomposition in Terrestrial Ecosystems. Annual Review of Ecology Evolution and Systematics 36, 191-218.

Hoorens, B., Aerts, R., Stroetenga, M., 2002. Litter quality and interactive effects in litter mixtures: more negative interactions under elevated CO_2? Journal of Ecology 90, 1009-1016.

Iqbal, A., Beaugrand, J., Garnier, P., Recous, S., 2013. Tissue density determines the water storage characteristics of crop residues. Plant and Soil 367, 285-299.

Jensen, L.S., Salo, T., Palmason, F., Breland, T.A., Henriksen, T.M., Stenberg, B., Pedersen, A., Lundström, C., Esala, M., 2005. Influence of biochemical quality on C and N mineralisation from a broad variety of plant materials in soil. Plant and Soil 273, 307-326.

Jung, J.Y., Lal, R., Ussiri, D.A.N., 2011. Changes in CO_2, ^{13}C abundance, inorganic nitrogen, β-glucosidase, and oxidative enzyme activities of soil during the decomposition of switchgrass root carbon as affected by inorganic nitrogen additions. Biology and Fertility of Soils 47, 801-813.

Keeney, D.R., Nelson, D.W., 1982. Nitrogen in organic forms. In: Page, A.L., (Eds.) Methods of Soil Analysis. Part 2. Agronomy monograph, second ed. ASA and SSSA, Madison, WI, pp. 643-698.

King, H.G., Health, G.W., 1967. The chemical analyses of small samples of leaf material and the relationship between the disappearance and composition of leaves. Pedobiologia 7, 192-197

Lal R., Reicosky D.L., Hanson J.D., 2007 Evolution of the plow over 10,000 years and the rationale for no-till farming. Soil & Tillage Research 93, 1-12.

Li, L.J., Han, X.Z., You, M.Y., Yuan, Y.R., Ding, X.L., Qiao, Y.F., 2013a. Carbon and nitrogen mineralization patterns of two contrasting crop residues in a Mollisol: Effects of residue type and placement in soils. European Journal of Soil Biology 54, 1-6.

Li, D., Peng, S., Chen, B., 2013b. The effects of leaf litter evenness on decomposition depend on which plant functional group is dominant. Plant and Soil 365, 255-266.

Liu, P., Sun, O.J., Huang, J., Li, L., Han, X., 2007. Nonadditive effects of litter mixtures on decomposition and correlation with initial litter N and P concentrations in grassland plant species of northern China. Biology and Fertility of Soils 44, 211-216.

Lummer, D., Scheu, S., Butenschoen, O., 2012. Connecting litter quality, microbial community and nitrogen transfer mechanisms in decomposing litter mixtures. Oikos 121, 1649-1655.

Makkonen, M., Berg, M.P., Van Logtestijn, R., S.P., Van Hal, J.R., Aerts, R., 2013. Do physical plant litter traits explain non-additivity in litter mixtures? A test of the improved microenvironmental conditions theory. Oikos 122, 987-997.

Pavoine, S., Vallet, J., Dufour, A.B., Gachet, S.; Daniel H., 2009. On the challenge of treating various types of variables: application for improving the measurement of functional diversity. Oikos 118, 391-402.

Quemada, M., Cabrera, M.L., 1995. Carbon and nitrogen mineralized from leaves and stems of four cover crops. Soil Science Society of America Journal 59, 471-477.

Recous, S., Robin, D., Darwis, D., Mary, B., 1995. Soil inorganic N availability: effect on maize residue decomposition. Soil Biology & Biochemistry 27, 1529-1538.

Reinertsen, S.A., Elliott, L.F., Cochran, V.L., Campbell, G.S., 1984. The role of available C and N in determining the rate of wheat straw decomposition. Soil Biology & Biochemistry 16, 459-464.

Robinson, C.H., Kirkham, J.B., Littlewood, R., 1999. Decomposition of root mixtures from high arctic plants: a microcosm study. Soil Biology & Biochemistry 31, 1101-1108.

Tian, G., Brussaard, L., Kang, B.T., 1995. An index for assessing the quality of plant residues and evaluating their effects on soil and crop in the sub-humid tropics. Applied Soil Ecology 2, 25-32.

Thippayarugs, S., Toomsan, B., Vityakon, P., Limpinuntana, V., Pananothai, A., Cadish, G., 2008. Interactions in decomposition and mineralization between tropical legume residue components. Agroforestry Systems 72, 137-148.

Trinsoutrot, I., Recous, S., Bentz, B., Linères, M., Chèneby, D., Nicolardot, B., 2000. Biochemical quality of crop residues and carbon and nitrogen mineralization kinetics under nonlimiting nitrogen conditions. Soil Science Society of America Journal 64, 918-926.

Van Soest, P.J., 1963. Use of detergents in the analysis of fibrous feeds. I. Preparation of fiber residues of low nitrogen content. Journal of the Association of Official Analytical Chemists 46, 825-835.

Zeng, D.H., Mao, R., Chang, S.X., Li, L.J., Yang, D., 2010. Carbon mineralization of tree leaf litter and crop residues from poplar-based agroforestry systems in Northeast China: A laboratory study. Applied Soil Ecology 44, 133-137.

Table 1: Description, agricultural use of crops used and proportion of their leaves and stems determined in field (% total dry matter).

Latin name	English name	Family	Plant use	% Leaves[a]	% Stems	REF[b]
Phaseolus vulgaris	Bean	Fabaceae	Main crop	43 ± 3.1	57 ± 3.7	1
Glycine max	Soybean	Fabaceae	Main crop	38 ± 3.2	62 ± 2.1	2
Zea mays	Maize	Poaceae	Main crop	26 ± 3.6	74 ± 3.9	3
Helianthus annuus	Sunflower	Asteraceae	Main crop	39 ± 4.2	61 ± 5.1	4
Crotalaria juncea	Sunn hemp	Fabaceae	Cover crop	19 ± 2.3	81 ± 4.6	5
Canavalia ensiformis	Jack bean	Fabaceae	Cover crop	72 ± 4.9	28 ± 3.8	6
Stizolobium niveum	Gray mucuna	Fabaceae	Cover crop	42 ± 3.6	58 ± 2.9	7
Pennisetum glaucum	Millet	Poaceae	Cover crop	32 ± 4.1	68 ± 2.5	8
Sorghum bicolor	Sorghum	Poaceae	Main crop	55 ± 3.5	45 ± 4.3	9
Crotalaria spectabilis	Showy rattlebox	Fabaceae	Cover crop	30 ± 3.1	70 ± 3.1	10
Avena strigosa	Black oat	Poaceae	Cover crop	48 ± 2.0	52 ± 2.7	11
Vicia sativa	Vetch	Fabaceae	Cover crop	62 ± 1.8	38 ± 3.5	12
Triticum aestivum	Wheat	Poaceae	Main crop	42 ± 4.1	58 ± 3.3	13
Raphanus sativus oleiferus	Oilseed radish	Brassicaceae	Cover crop	38 ± 2.2	62 ± 3.3	14
Secale cereale	Rye	Poaceae	Main crop	29 ± 3.1	71 ± 4.2	15
Pisum arvensis	Pea	Fabaceae	Cover crop	68 ± 2.4	32 ± 2.4	16
Triticosecale rimpaui	Triticale	Poaceae	Main crop	44 ± 2.8	56 ± 3.9	17
Brassica napus oleifera	Oilseed rape	Brassicaceae	Main crop	28 ± 3.6	72 ± 2.8	18
Hordeum vulgare	Barley	Poaceae	Main crop	50 ± 3.3	50 ± 2.0	19
Lolium multiflorum	Ryegrass	Poaceae	Cover crop	37 ± 3.2	63 ± 2.9	20
Ricinus communis	Castor bean	Euphorbiaceae	Main crop	52 ± 5.1	48 ± 4.4	21
Cajanus cajan	Dwarf pigeonpea	Fabaceae	Cover crop	34 ± 3.2	66 ± 3.7	22
Lupinus angustifolius	Blue lupine	Fabaceae	Cover crop	57 ± 2.7	43 ± 4.3	23
Lupinus albescens	Native lupine	Fabaceae	Cover crop	51 ± 3.7	49 ± 4.1	24
Oryza sativa	Rice	Poaceae	Main crop	50 ± 2.9	50 ± 3.4	25

[a] Proportion of leaves and stems in the total dry matter of shoots determined at flowering for cover crops and harvest for main crops. Means (n=3) ± standard deviation (SD).
[b] Reference.

Table 2: Parameters of chemical composition, carbon mineralization and nitrogen mineralization of different types of residues used in this study (minimum, maximum and mean values).

Parameter	Leaves			Stems			Mixture		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Chemical fraction (g kg^{-1} DM)									
SOL[a]	167	817	546	111	442	249	141	585	373
HEM	3	406	143	60	331	197	60	323	170
CEL	139	443	258	351	614	452	243	511	375
LIG	16	137	53	30	198	102	33	143	82
C	391	486	432	387	458	442	394	466	441
N	5.6	51.8	26.1	2.6	24.7	9.7	4.3	40.3	17.0
Csw	33.1	326.3	141.6	28.3	227.8	83.3	40.8	252.5	110.3
Nsw	3.4	35.8	12.6	1.4	29.3	6.8	2.9	34.0	9.5
POL	4.3	78.2	30.2	2.8	17.3	9.6	6.4	49.8	20.1
C and N mineralization									
C_0[b] (%)	46.9	64.3	56.0	25.8	56.9	46.1	41.1	60.7	51.0
k[c] (day^{-1})	0.022	0.128	0.063	0.012	0.109	0.039	0.021	0.104	0.050
C min 120 d (% added C)	43.9	67.1	58.0	26.0	61.5	46.7	39.4	63.4	52.6
Net N min 120 d (mg N kg^{-1} soil)	-20.0	140.8	45.3	-38.0	52.7	-7.7	-26.3	79.1	13.1

[a] SOL: Soluble fraction (Van Soest); HEM: Hemicellulose; CEL: Cellulose; LIG: Lignin; C: Total organic carbon; N: Total nitrogen; Csw: Water-soluble carbon; Nsw: Water-soluble nitrogen; POL: Soluble polyphenols; DM: Dry matter.

[b] Potentially mineralizable C pool.

[c] Mineralization constant rate.

Table 3: Multiple regression analyses with the relationships between C mineralization and the chemical composition of residues.

Residue		k (day^{-1})[b]	r^2[c]	C_o (%)[d]	r^2
Leaf	All[a]	+0.57 N -0.49 Csw +0.80 Nsw	0.78***	+0.73 N	0.79***
	Fabaceae	-2.15 SOL -0.82 HEM -1.64 CEL +1.04 N +1.18 POL	0.91***	-1.23 HEM -0.88 CEL +0.41 N +1.10 Csw	0.95***
	Poaceae	-0.28 HEM -0.45 CEL +0.61 N	0.97***	+0.69 SOL	0.78***
Stem	All	-0.32 HEM -0.40 CEL +0.52 N	0.80***	+0.54 CEL +0.76 Csw	0.42***
	Fabaceae	+0.84 SOL	0.70***	+1.76 SOL +1.34 CEL	0.60***
	Poaceae	-0.81 LIG	0.66***	+0.68 Csw	0.46***
Mixture	All	-0.80 HEM -0.48 CEL -0.30 LIG	0.78***	+0.74 N	0.54***
	Fabaceae	-0.73 HEM	0.53***	-0.63 HEM -1.1 CEL -0.95 POL	0.87***
	Poaceae	+0.90 N	0.82***	+0.71 N	0.92***

[a] Residues from 5 botanical families (Fabaceae, Poaceae, Brassicaceae, Asteraceae and Euphorbiaceae).
[b] Mineralization constant rate.
[c] Coefficient of determination. ***P<0.001.
[d] Potentially mineralizable C pool.

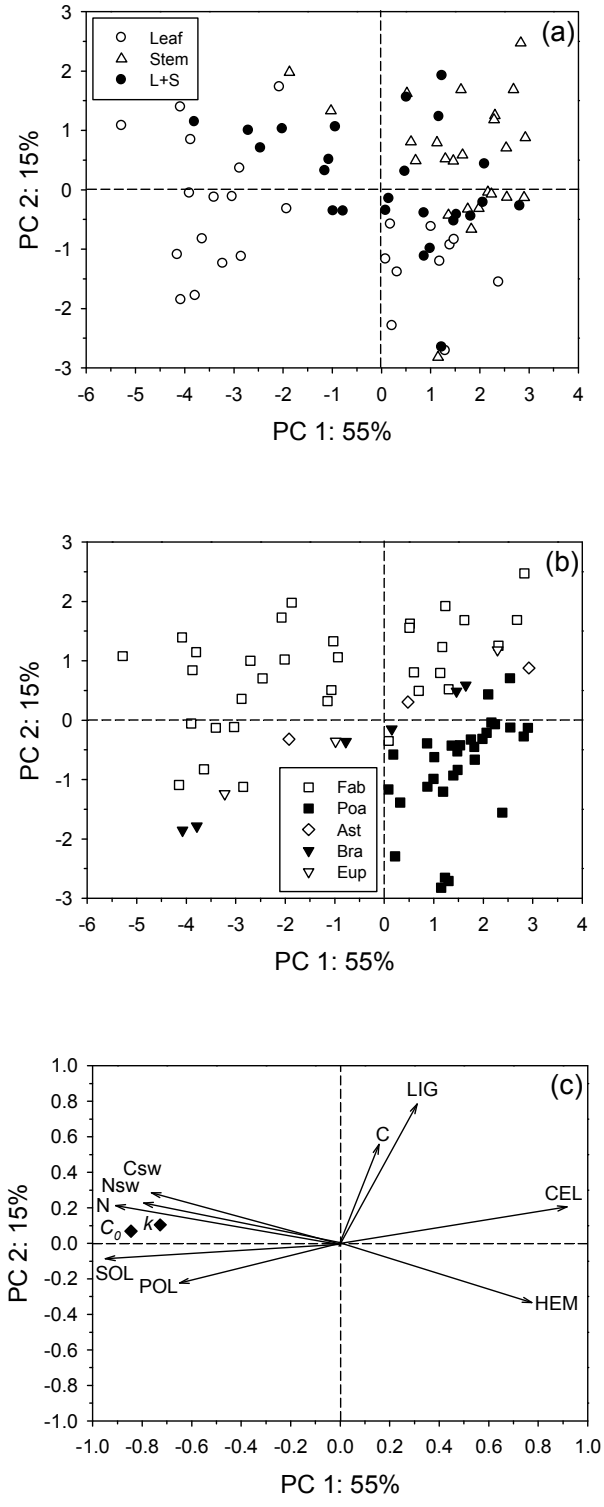

Fig. 1: Factorial map (PC 1 x PC 2) (a, b) and correlation circle (c) obtained from principal component analysis (PCA) performed on biochemical properties (i.e., Van Soest fractions, total N, total C, total N, water-soluble fractions and polyphenols) of leaves, stems and mixtures of leaves + stems (L+S). Mineralization constant (k) and mineralizable C pool (C_0) were added as supplementary variables, i.e., not included in the ordination of species. Fab: Fabaceae; Poa: Poaceae; Ast: Asteraceae; Bra: Brassicaceae; Eup: Euphorbiaceae.

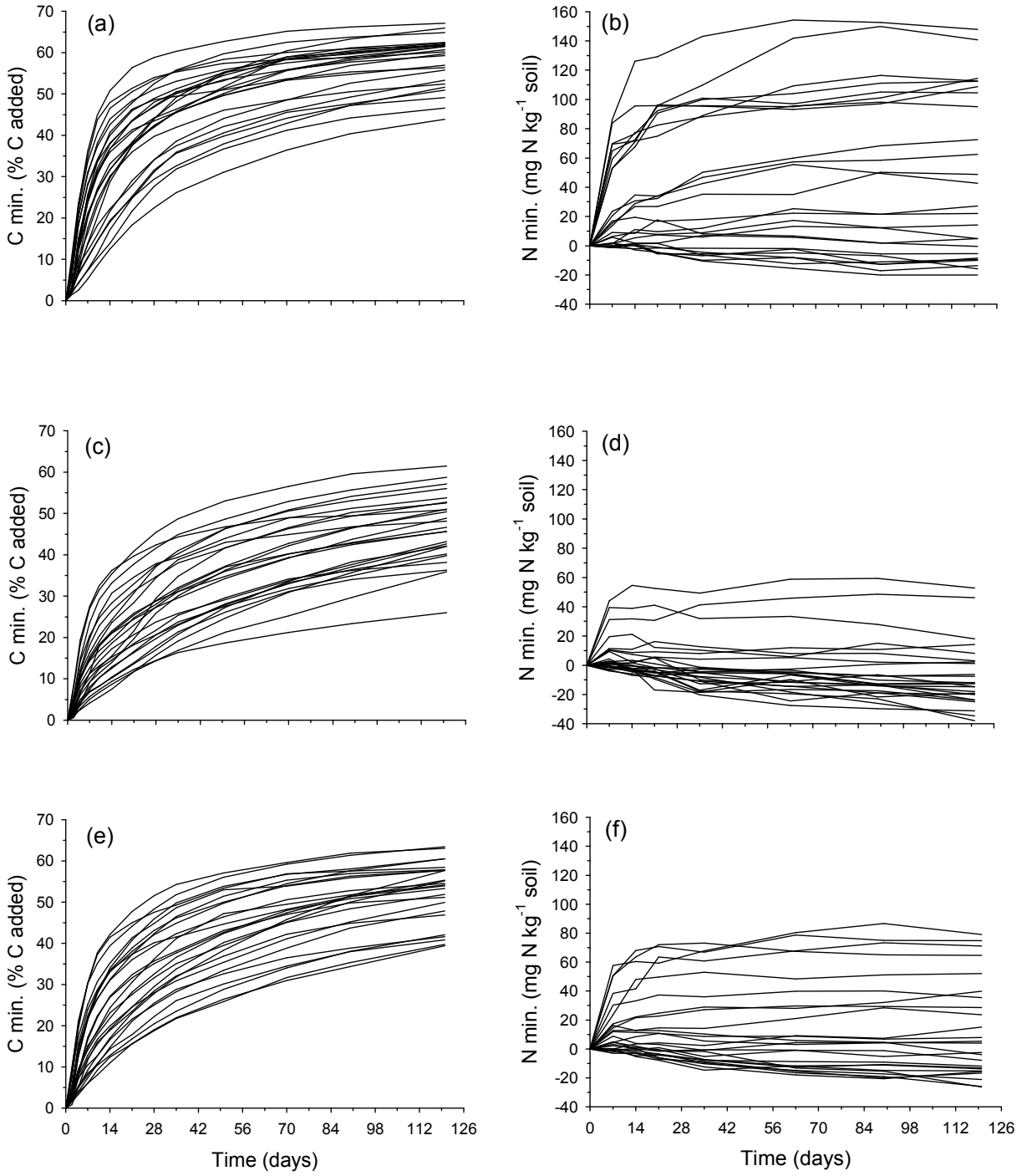

Fig. 2: Cumulative CO_2 mineralization (a, c, e) and net changes in soil mineral N contents (b, d, f) for leaves, stems and mixtures (leaf + stem), during decomposition in soil at 25°C for 120 days, respectively. Net mineralization was calculated by the difference between amended and non-amended control treatments. Values are the mean of 3 replicates.

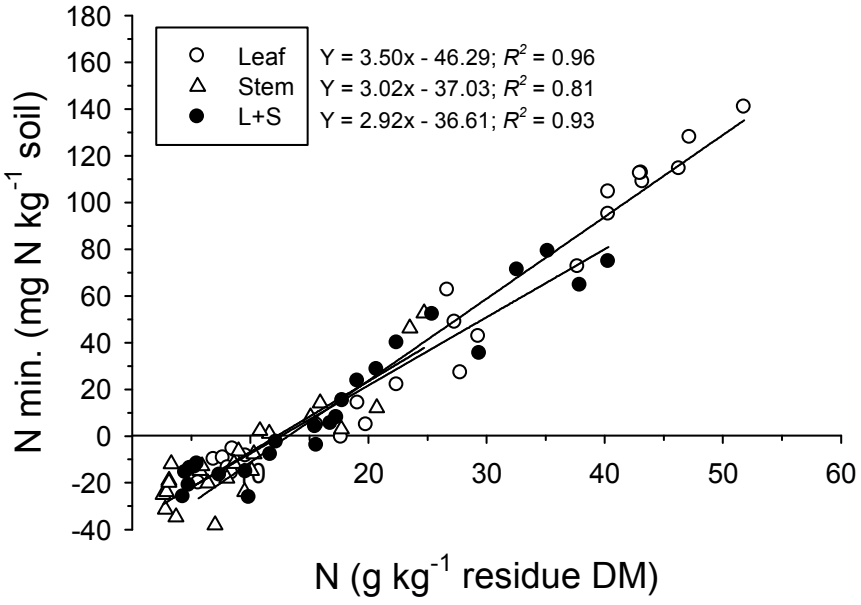

Fig. 3: Relationships between net N mineralized in soil after 120 days of incubation and initial total N content in the dry matter (DM) of the leaves, stems and mixtures of leaves + stems (L+S) for 25 crop species. Lines indicate the 95% confidence intervals for the difference of means (P<0.001) between leaves, stems and leaf and stem mixtures (L+S).

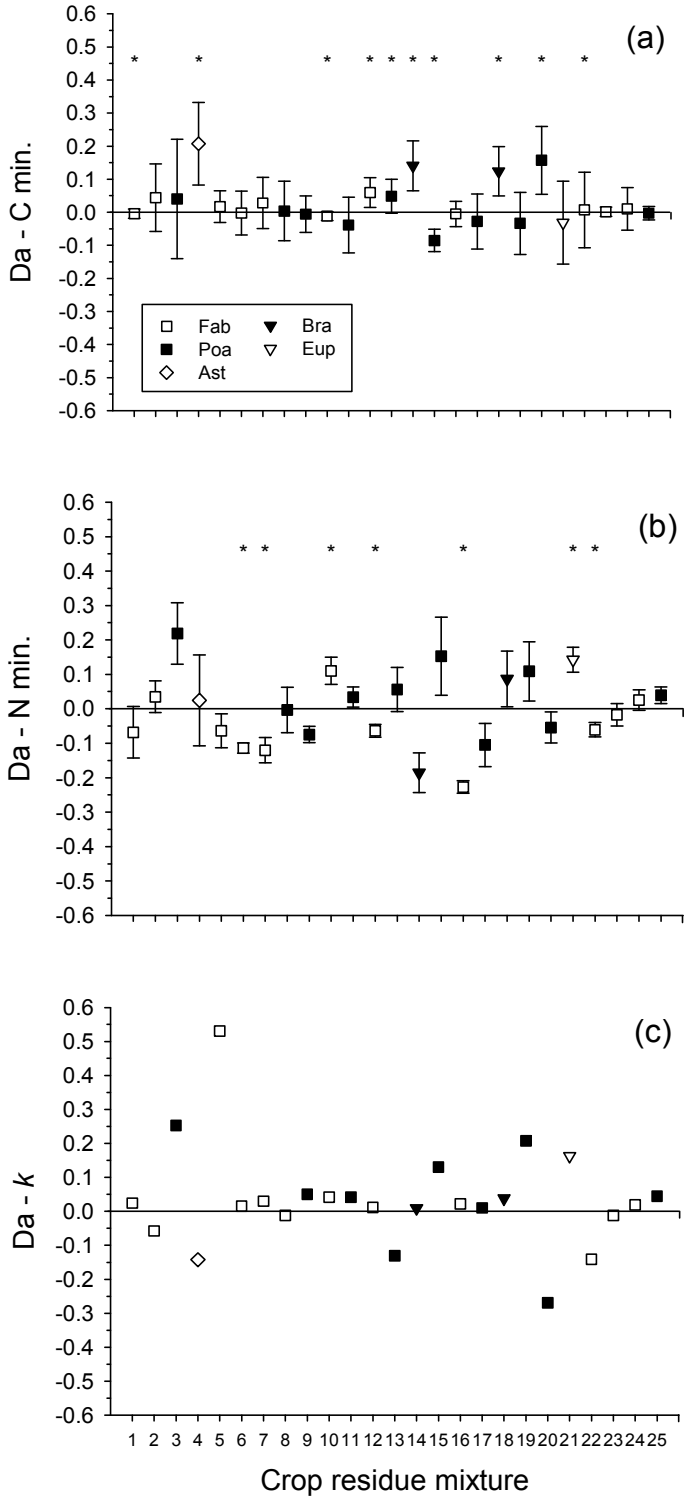

Fig. 4: Average deviation from additivity (Da) calculated for C mineralized in residue (a), net N mineralization in soil (b) and mineralization constant *k* (c) in 25 litter mixtures after 120 days of incubation. Asterisks indicate significant difference of means according to Tukey's test ($P<0.05$). Fab: Fabaceae; Poa: Poaceae; Ast: Asteraceae; Bra: Brassicaceae; Eup: Euphorbiaceae. See Table 1 for the litter mixture species studied. Bars indicate the standard deviation of the means for mineralized C and N.

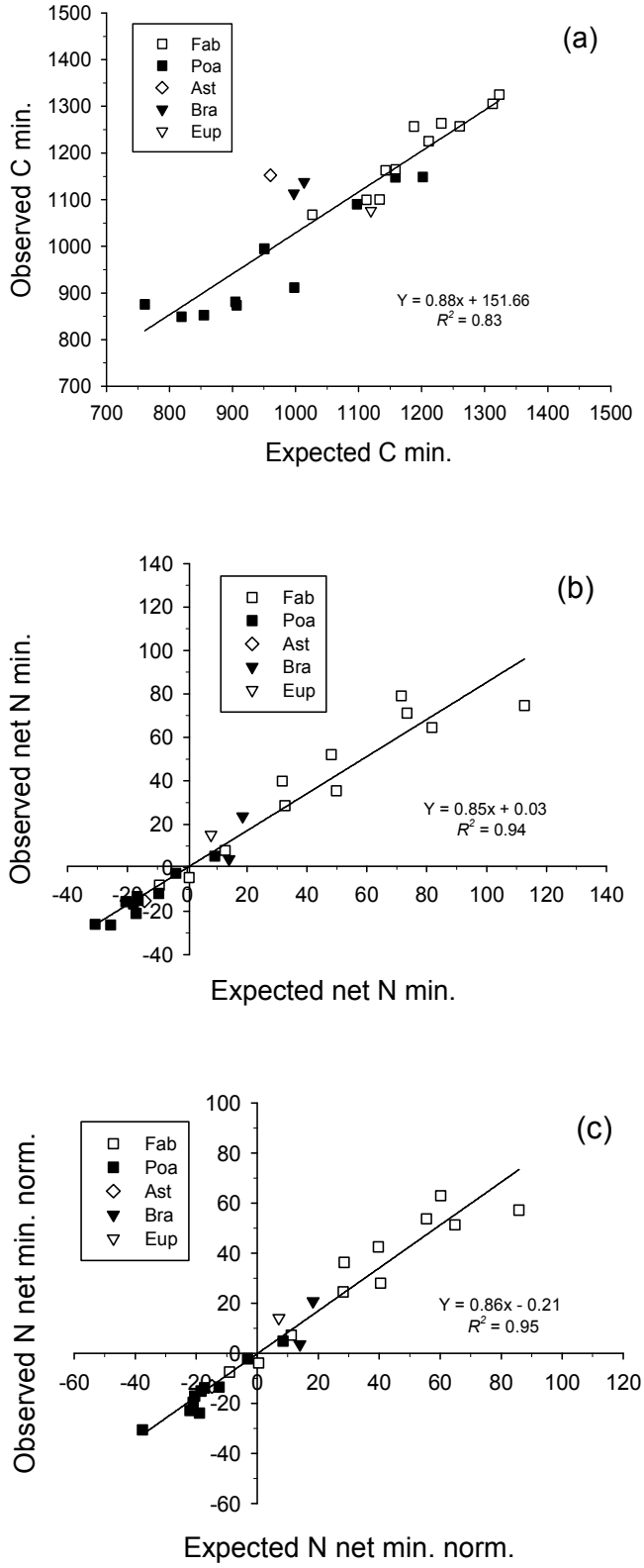

Fig. 5: Observed vs. expected C mineralization (mg C-CO_2 kg^{-1} soil) (a), net N mineralization (b) and net N mineralization after normalization (mg N kg^{-1} soil) (c). Normalization consisted of expressing net N mineralization per unit of C mineralized. Fab: Fabaceae; Poa: Poaceae; Ast: Asteraceae; Bra: Brassicaceae; Eup: Euphorbiaceae.

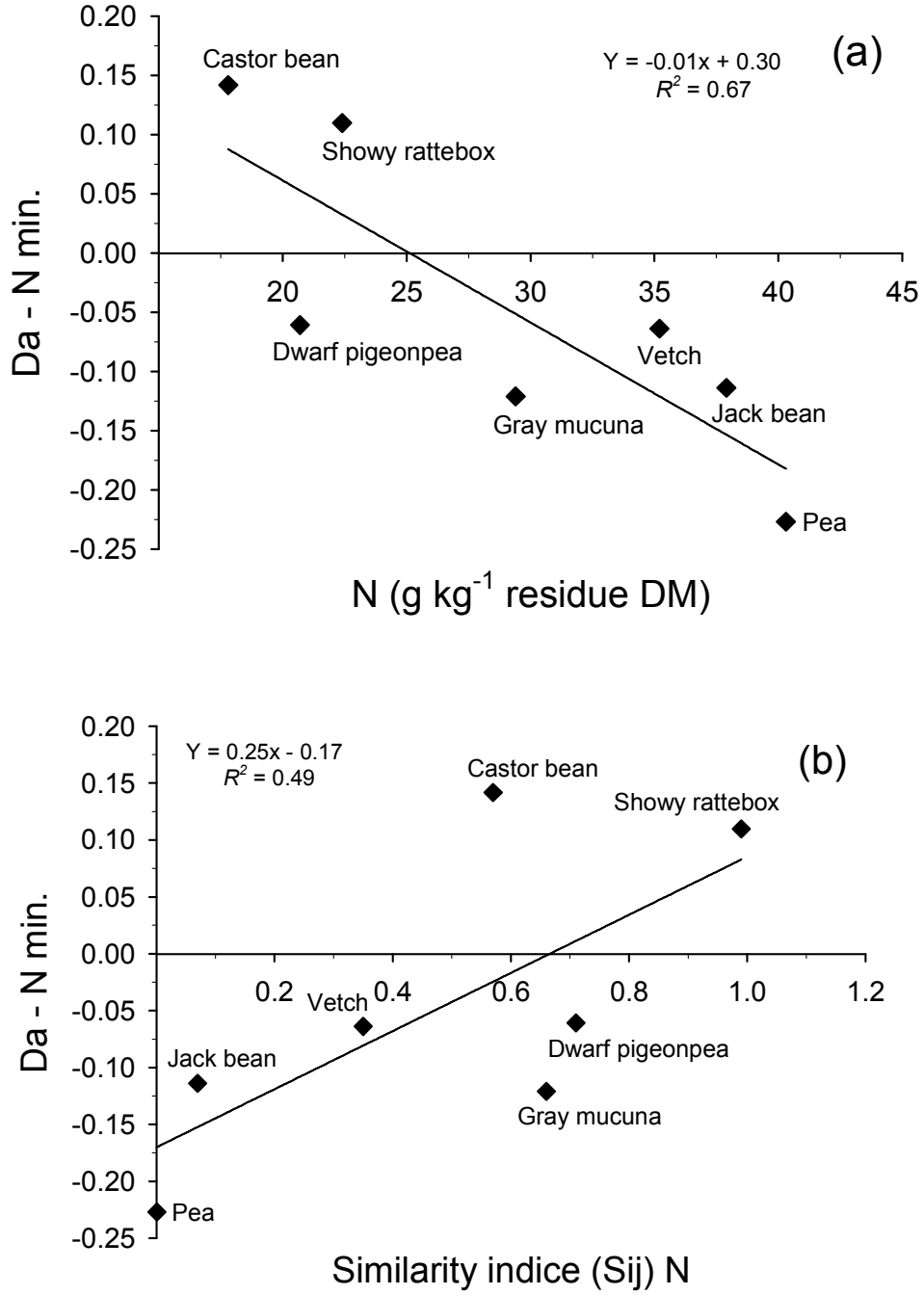

Fig. 6: Relationships between deviations from additivity (Da) calculated for net N mineralization and mixture initial total N (a) and mixture heterogeneity coefficient of similarity for total N (S_{ijk}) (b). The relationship was established for 7 mixtures (leaf + stem) showing significant synergistic (positive Da) or antagonistic (negative Da) non-additive effects for N mineralization. S_{ijk} for total N was a similarity coefficient calculated according to Gower and Legendre (1986).

Supplementary data A: Coefficient of similarity between leaf and stem calculated for each chemical characteristics and each species using Gower's coefficient (S_{ijk}) and aggregated similarity coefficient (S_{ij}) calculated as the mean of S_{ijk} for the whole set of characteristics.

Species	Similarity coefficient (S_{ijk})									
	SOL[a]	HEM	CEL	LIG	C	N	Csw	Nsw	POL	**S_{ij}**
1 – Bean	0.09	0.28	0.16	0.17	0.29	0.52	0.24	0.38	0.54	**0.30**
2 – Soybean	0.00	0.29	0.06	0.00	0.80	0.67	0.11	0.37	0.58	**0.32**
3 – Maize	0.95	0.94	0.96	0.74	0.27	0.95	0.76	0.99	0.97	**0.84**
4 – Sunflower	0.47	0.89	0.16	0.92	0.66	0.54	0.37	0.62	0.00	**0.51**
5 – Sunn hemp	0.04	0.27	0.00	0.42	0.20	0.13	0.29	0.44	0.75	**0.28**
6 – Jack bean	0.31	0.55	0.28	0.48	0.44	0.37	0.00	0.83	0.81	**0.45**
7 – Gray mucuna	0.44	0.90	0.33	0.38	0.34	0.20	0.25	0.94	0.91	**0.52**
8 – Millet	0.72	0.91	0.72	0.60	0.00	0.70	0.95	0.85	0.85	**0.70**
9 – Sorghum	0.80	0.89	0.79	0.82	0.95	0.62	0.90	0.90	0.62	**0.81**
10 – Showy rattlebox	0.25	0.62	0.16	0.42	0.93	0.39	0.74	0.94	0.65	**0.56**
11 – Black oat	0.79	0.85	0.77	0.90	0.85	0.80	0.41	0.95	0.92	**0.80**
12 – Vetch	0.59	0.65	0.64	0.59	0.76	0.45	0.58	0.84	0.76	**0.65**
13 – Wheat	0.98	0.79	0.96	0.87	0.56	0.89	0.58	0.82	0.72	**0.80**
14 – Oilseed radish	0.17	0.42	0.22	0.18	0.20	0.41	0.16	0.00	0.73	**0.28**
15 – Rye	0.80	0.98	0.78	0.67	0.73	0.86	0.95	0.78	0.77	**0.81**
16 – Pea	0.61	0.97	0.53	0.44	0.88	0.24	0.19	0.66	0.79	**0.59**
17 – Triticale	0.95	0.39	0.65	0.84	0.85	0.81	0.80	0.78	0.97	**0.78**
18 – Oilseed rape	0.06	0.00	0.28	0.14	0.54	0.60	0.39	0.45	0.72	**0.35**
19 – Barley	0.99	0.81	0.99	0.78	0.59	0.86	0.62	0.78	0.93	**0.82**
20 – Ryegrass	0.86	0.79	0.94	0.84	0.44	0.85	0.70	0.82	0.95	**0.80**
21 – Castor bean	0.13	0.41	0.03	0.54	0.54	0.38	0.68	0.85	0.20	**0.42**
22 – Dwarf pigeonpea	0.24	0.65	0.02	0.73	0.32	0.08	0.55	0.73	0.64	**0.44**
23 – Blue lupine	0.45	0.49	0.29	0.78	0.93	0.00	0.33	0.23	0.68	**0.46**
24 – Native lupine	0.37	0.39	0.48	0.43	0.98	0.17	0.35	0.60	0.64	**0.49**
25 – Rice	0.95	0.95	0.98	0.90	0.90	0.94	0.83	0.97	0.92	**0.93**

[a] SOL: Soluble fraction (Van Soest); HEM: Hemicellulose; CEL: Cellulose; LIG: Lignin; C: Total organic carbon; N: Total nitrogen; Csw: Water-soluble carbon; Nsw: Water-soluble nitrogen; POL: Soluble polyphenols.

Supplementary data B: Weighted coefficient of similarity between leaf and stem calculated for each chemical characteristics and each species using Gower's coefficient weighted (S_{pij}) and aggregated similarity coefficient (S_{ij}) calculated for the whole set of characteristics. The weighted coefficients were calculated taking into account the proportion of leaf and stem masses in the mixtures.

Species	Similarity coefficient (S_{pij})									
	SOL[a]	HEM	CEL	LIG	C	N	Csw	Nsw	POL	**S_{ij}**
1 – Bean	0.51	0.46	0.52	0.29	0.74	0.78	0.82	0.73	0.56	**0.60**
2 – Soybean	0.52	0.37	0.39	0.00	0.61	0.91	0.79	0.77	0.67	**0.56**
3 – Maize	0.87	0.00	0.47	0.52	0.21	0.95	0.92	0.89	0.88	**0.63**
4 – Sunflower	0.79	0.82	0.39	0.75	0.65	0.79	0.84	0.83	0.09	**0.66**
5 – Sunn hemp	0.97	0.19	0.00	0.12	0.00	1.00	0.76	0.98	0.96	**0.55**
6 – Jack bean	0.00	0.96	0.99	0.97	0.36	0.07	0.01	0.46	0.53	**0.48**
7 – Gray mucuna	0.78	0.81	0.59	0.43	0.80	0.66	0.86	0.97	1.00	**0.77**
8 – Millet	0.98	0.29	0.54	0.53	0.39	0.97	0.75	0.99	1.00	**0.71**
9 – Sorghum	0.79	0.87	0.99	0.92	0.85	0.70	0.91	0.89	0.47	**0.82**
10 – Showy rattlebox	0.94	0.55	0.31	0.32	0.39	0.99	0.96	0.84	0.94	**0.69**
11 – Black oat	0.88	0.84	0.86	0.91	0.93	0.89	0.78	0.99	0.92	**0.89**
12 – Vetch	0.41	0.93	1.00	0.92	0.60	0.35	0.46	0.57	0.55	**0.64**
13 – Wheat	0.86	0.90	0.84	0.81	0.73	0.96	0.94	0.94	0.79	**0.86**
14 – Oilseed radish	0.70	0.49	0.48	0.26	0.60	0.76	0.86	0.59	0.82	**0.62**
15 – Rye	0.93	0.24	0.49	0.41	0.32	0.99	0.74	0.98	0.97	**0.67**
16 – Pea	0.26	0.84	0.96	0.90	0.43	0.00	0.00	0.00	0.56	**0.44**
17 – Triticale	0.98	0.86	0.73	0.82	0.80	0.90	0.99	0.90	0.99	**0.89**
18 – Oilseed rape	0.85	0.04	0.36	0.10	0.32	0.90	0.98	0.85	0.93	**0.59**
19 – Barley	0.99	0.88	0.99	0.85	0.97	0.91	0.84	0.86	0.91	**0.91**
20 – Ryegrass	0.96	0.41	0.70	0.73	0.55	0.96	0.99	0.95	0.85	**0.79**
21 – Castor bean	0.33	0.63	0.60	0.74	0.97	0.57	0.85	0.89	0.00	**0.62**
22 – Dwarf pigeonpea	0.73	0.45	0.30	0.42	0.52	0.71	0.94	1.00	0.77	**0.65**
23 – Blue lupine	0.43	0.75	0.77	0.70	0.79	0.20	0.56	0.37	0.53	**0.57**
24 – Native lupine	0.52	0.61	0.78	0.64	0.97	0.44	0.72	0.73	0.56	**0.66**
25 – Rice	0.96	0.97	0.99	0.93	0.99	0.96	0.93	0.98	0.90	**0.96**

[a] SOL: Soluble fraction (Van Soest); HEM: Hemicellulose; CEL: Cellulose; LIG: Lignin; C: Total organic carbon; N: Total nitrogen; Csw: Water-soluble carbon; Nsw: Water-soluble nitrogen; POL: Soluble polyphenols.

Supplementary data C: Cumulative residue-C mineralized and kinetic coefficients calculated from a simple exponential decay function for leaves, stems and mixture (leaf + stem) of plant residues incubated at 25ºC during 120 days.

Species	Leaves			Stems			Leaf + Stem		
	C min. measured[a] %	C_0[b] %	k[c] day^{-1}	C min. measured %	C_0 %	k day^{-1}	C min. measured %	C_0 %	k day^{-1}
1 – Bean	61.5 Ba*	57.5	0.083	45.7 Cc	42.0	0.047	54.6 Bb	50.6	0.064
2 – Soybean	60.7 Ba	57.4	0.053	40.2 Db	41.4	0.024	49.9 Cab	47.8	0.033
3 – Maize	43.9 Ea	46.9	0.022	35.9 Fa	39.9	0.016	39.4 Da	41.1	0.022
4 – Sunflower	57.0 Ca	56.1	0.047	42.1 Db	56.1	0.012	57.7 Aa	60.7	0.022
5 – Sunn hemp	59.3 Ca	56.4	0.074	52.7 Ba	54.9	0.027	55.3 Ba	52.9	0.055
6 – Jack bean	62.4 Ba	59.4	0.064	56.0 Aa	54.2	0.043	60.5 Aa	58.2	0.059
7 – Gray mucuna	61.5 Ba	56.5	0.066	57.1 Aa	53.1	0.051	60.5 Aa	56.0	0.059
8 – Millet	65.9 Aa	63.6	0.046	51.0 Bb	53.7	0.023	55.2 Bb	54.1	0.030
9 – Sorghum	52.4 Da	50.3	0.058	50.4 Ba	47.6	0.037	51.2 Ba	49.0	0.051
10 – Showy rattlebox	56.4 Ca	54.1	0.088	53.8 Ba	49.6	0.061	53.9 Ba	49.2	0.072
11 – Black oat	61.9 Ba	61.6	0.045	46.6 Cc	44.8	0.036	54.2 Bb	53.2	0.042
12 – Vetch	62.1 Ba	57.8	0.099	50.9 Bb	47.6	0.109	58.4 Aa	54.6	0.104
13 – Wheat	53.3 Da	51.3	0.038	42.7 Db	40.7	0.028	47.9 Cab	47.5	0.028
14 – Oilseed radish	60.2 Ca	57.6	0.128	45.6 Cb	43.3	0.043	57.7 Aa	54.7	0.076
15 – Rye	51.6 Da	50.5	0.031	42.2 Db	49.4	0.016	42.0 Db	43.5	0.023
16 – Pea	64.8 Aa	61.8	0.086	61.5 Aa	56.9	0.071	63.4 Aa	58.5	0.083
17 – Triticale	49.1 Da	49.3	0.035	36.3 Eb	39.1	0.022	40.9 Db	41.3	0.028
18 – Oilseed rape	61.6 Ba	59.1	0.108	48.1 Bb	47.2	0.049	57.8 Aab	55.0	0.068
19 – Barley	46.6 Ea	48.5	0.028	39.8 Db	39.8	0.025	41.6 Da	41.8	0.032
20 – Ryegrass	51.0 Da	52.3	0.030	26.0 Fc	25.8	0.028	39.8 Db	41.8	0.021
21 – Castor bean	62.5 Ba	60.4	0.048	43.2 Db	39.7	0.034	53.3 Bab	50.3	0.048
22 – Dwarf pigeonpea	59.8 Ca	54.7	0.069	48.9 Bb	45.3	0.035	51.9 Bab	49.3	0.040
23 – Blue lupine	67.1 Aa	64.3	0.109	58.7 Ab	53.8	0.065	63.0 Aab	59.4	0.089
24 – Native lupine	61.8 Ba	57.4	0.078	52.6 Bb	48.9	0.051	57.7 Aab	54.4	0.066
25 – Rice	55.8 Ca	54.7	0.035	38.2 Ec	38.4	0.032	46.9 Cb	46.6	0.035

[a] The % of added C mineralized from residues plant after 120 days. [b] Potentially mineralizable C pool. [c] Mineralization constant rate. * Mean values with different large letters in the column are different by Scott-Knott test (P<0.05) and small letters in the lines by Tukey test (P<0.05).

Supplementary data D: Cumulative CO_2-C evolved and soil mineral N of mixtures (leaf + stem residues) of 25 crop species, observed at day 14 and day 120 of incubation, and expected values calculated using results of single leaf and stem treatments.

Species	Carbon min. (% added C)				Nitrogen min. (mg N kg^{-1} soil)			
	Measured	Expected[a]	Measured	Expected	Measured	Expected	Measured	Expected
	14 days		120 days		14 days		120 days	
1 – Bean	625	634 ns	1100	1133 *	22.9	25.8 ns	60.9	65.5 ns
2 – Soybean	412	428 ns	1068	1027 ns	15.1	15.2 ns	45.1	43.6 ns
3 – Maize	237	220 ns	849	819 ns	11.3	12.8 ns	26.9	22.1 ns
4 – Sunflower	274	227 *	1152	960 *	15.5	16.1 ns	37.7	37.3 ns
5 – Sunn hemp	458	513 ns	1163	1142 ns	31.6	33.2 ns	65.1	69.7 ns
6 – Jack bean	706	691 ns	1257	1260 ns	82.4	93.7 *	133.6	150.8 *
7 – Gray mucuna	690	658 ns	1264	1231 ns	52.1	66.8 *	104.5	118.9 *
8 – Millet	432	404 ns	1148	1158 ns	18.9	24.0 ns	42.8	43.0 ns
9 – Sorghum	578	556 ns	1090	1097 ns	24.0	26.7 ns	48.3	52.2 ns
10 – Showy rattlebox	681	688 ns	1100	1112 *	34.7	27.9 *	82.9	74.7 *
11 – Black oat	516	524 ns	1149	1202 ns	12.8	11.7 ns	39.6	38.3 ns
12 – Vetch	893	843 *	1257	1188 *	71.1	77.3 *	121.2	129.5 *
13 – Wheat	345	355 ns	995	951 *	5.5	5.6 ns	28.3	26.8 ns
14 – Oilseed radish	709	591 *	1113	997 *	14.7	18.9 ns	46.1	56.7 ns
15 – Rye	278	308 *	911	998 *	13.6	13.0 ns	38.9	34.0 ns
16 – Pea	811	829 ns	1305	1312 ns	83.9	98.3 *	129.2	167.1 *
17 – Triticale	309	306 ns	881	904 ns	16.1	15.2 ns	33.4	37.4 ns
18 – Oilseed rape	640	586 ns	1138	1014 *	28.8	32.1 ns	78.1	72.2 ns
19 – Barley	309	277 *	873	907 ns	10.7	9.2 ns	37.6	34.1 ns
20 – Ryegrass	274	255 *	876	761 *	7.3	9.3 ns	39.0	41.3 ns
21 – Castor bean	543	537 ns	1076	1119 ns	16.1	18.2 ns	58.2	50.9 *
22 – Dwarf pigeonpea	551	582 ns	1165	1159 *	34.2	37.8 *	71.6	76.2 *
23 – Blue lupine	886	883 ns	1325	1323 ns	53.5	74.5 *	121.9	124.2 ns
24 – Native lupine	709	688 *	1226	1211 ns	59.9	57.2 ns	102.9	100.3 ns
25 – Rice	343	334 ns	853	855 ns	8.9	10.7 ns	34.1	32.8 ns

[a] Expected values are calculated according to the proportion of leaves and stems in the mixtures (see Table 1). Asterisks in the lines indicate significant difference of means by Tukey test (P<0.05). Not significant (ns).

5 DISCUSSÃO GERAL

No presente trabalho buscou-se inicialmente realizar, no contexto agronômico, um estudo detalhado sobre acúmulo de MS, C e N nas raízes, determinar a relação R/PA e caracterização química de 27 espécies de culturas anuais de cinco famílias (Artigo 1). Posteriormente, em 20 espécies, o trabalho buscou estudar através de incubações de laboratório a relação da composição química das raízes com mineralização do C de modo a avaliar o potencial de contribuição das raízes para o C do solo (Artigo 2). Finalmente, em 25 espécies, o trabalho buscou estudar as interações de aditividade ou não aditividade relacionadas à decomposição na superfície do solo de misturas de folhas e talos com diferentes composições químicas (Artigo 3).

A realização do primeiro estudo (Artigo 1) surgiu da evidente necessidade da ampliação da fonte de informações sobre MS, composição química e acúmulo de C e N na MS de raízes de espécies de culturas anuais de diferentes famílias utilizadas em plantio direto nas condições de clima subtropical. Devido às restritas e limitadas informações disponíveis de raízes de culturas anuais, os trabalhos relacionados com entradas de C e N no solo por estimativas ou modelos matemáticos em clima subtropical, obriga comumente utilizar dados empíricos ou médios de literatura provenientes de condições de cultivo, solo e clima temperado (p.ex., LOVATO et al., 2004; DOS SANTOS et al., 2011). O segundo estudo também relacionado com raízes (Artigo 2) surgiu pelo mesmo motivo do Artigo 1, ou seja, o restrito e limitado número de trabalhos que avaliam a decomposição de raízes no solo (apenas 2%) (ZHANG et al., 2008).

Os resultados do primeiro estudo mostraram que a MS de raízes apresentou grande variação e foi dependente das famílias e das espécies de culturas anuais estudadas (Artigo 1; Tabela 3). Esses aspectos também foram observados em outros estudos com raízes, porém em clima temperado (BOLINDER et al., 1999; BOLINDER et al., 2002; BOLINDER et al., 2007). Em média, observou-se que a produção total de raízes e o acúmulo de C e N na MS das espécies de leguminosas (Fabaceae) foram inferiores àquelas obtidos com não leguminosas (principalmente composto por Poaceae) (Artigo 1; Tabela 3, Tabela 4). Assim, a relação R/PA média foi menor para espécies de leguminosas e maior para não leguminosas, porém com valores inferiores

(principalmente para Poaceae) aos encontrados em estudo de revisão com um grande número de espécies de plantas agrícolas de clima temperado no Canadá (BOLINDER et al., 2007). A utilização de resultados médios de literatura por não representar as condições de estudo, podem levar a resultados muito contrastantes, conforme mencionado no Artigo 1. Por exemplo, no trabalho de Santos et al. (2011) ao assumir um índice de relação R/PA de 0,43 para soja, 0,41 para milho e 0,30 para aveia preta, similares aos de Bolinder et al. (2007) estariam superestimando a contribuição das raízes para a adição e acúmulo de C no solo. No trabalho de Lovato et al. (2004), foi assumido um índice de 0,30 para a culturas anuais utilizadas em seu estudo, e assim, também poderiam estar superestimando a adição de C no solo via raízes.

As espécies de Poaceae apresentaram a maior quantidade de raízes finas se comparado às Fabaceae (Artigo 1; Tabela 3). A maior proporção da MS total produzida pelas raízes das plantas concentrou-se na camada próxima à superfície do solo e também da linha e/ou cova de semeadura para espécies de primavera/verão (Artigo 1; Figura 1). As propriedades físicas das raízes podem assumir grande relevância para a sustentabilidade do sistema de plantio direto. De acordo com Filho et al. (2004) devido que neste sistema sem revolvimento do solo e intenso tráfego de máquinas, a presença de poros com grande diâmetro a partir das raízes podem favorecer a fauna do solo, a infiltração de água e trocas de gases. Esses resultados estão relacionados à própria natureza da família das espécies que determina a produção e alocação da MS, tipologia, morfologia e composição química das raízes (ROUMET et al., 2008). Além disso, a concentração de raízes nas camadas superficiais, provavelmente esteja relacionada com a maior quantidade de poros e fissuras entre os elementos estruturais do solo em plantio direto, vias preferenciais ao crescimento de raízes no solo (FILHO et al., 2001). Assim, provavelmente a maior contribuição de C proveniente do sistema radicular será nas camadas superficiais e próximo a linha e/ou cova de semeadura. Ainda, as raízes podem ser uma importante fonte de C, N e outros nutrientes para os microorganismos decompositores do solo. A decomposição das raízes concentradas próximas à superfície do solo, geralmente pobres em N, podem estimular a imobilização de N no solo e influenciar o desenvolvimento das culturas em sucessão (principalmente Poaceae) pela limitação de N.

A composição química das raízes de espécies de Poaceae mostrou baixo conteúdo de lignina e raízes de Fabaceae apresentaram alto conteúdo de fração solúvel (Van Soest), N total, celulose e lignina (Artigo 2; Figura 1, Tabela 2). O fato das raízes

de Poaceae apresentarem baixo conteúdo de lignina pode estar relacionado com o estágio fenológico da coleta das raízes, conforme discutido no Artigo 2. De maneira geral, as raízes de espécies de Fabaceae mostraram a maior quantidade de C mineralizado (média de 48% do C adicionado) e menor para espécies de Poaceae (média 42%) (Artigo 2, Figura 2, Tabela 3). Os resultados mostraram que as diferenças na mineralização do C das raízes foram explicadas pelo conteúdo de celulose e hemicelulose das raízes. As raízes de espécies de Poaceae mostraram menor velocidade e mineralização cumulativa do C devido ao baixo conteúdo de celulose, N total e alto conteúdo de hemicelulose.

A partir dos resultados obtidos nos Artigos 1 e 2 pode-se inferir que algumas culturas anuais, principalmente Poaceae, devido à elevada produção de MS, raízes finas (Artigo 1) e/ou baixa mineralização do C no solo (Poaceae e algumas espécies de Fabaceae) (Artigo 2) podem provocar melhorias nas propriedades físicas, químicas e biológicas do solo. Segundo Rasse et al. (2005) as raízes de Poaceae crescem com maior facilidade no interior dos agregados, o C fica mais protegido da atividade microbiana e pode ser mais facilmente estabilizado no solo. Além disso, as raízes finas são fisiologicamente mais ativas e tem grande potencial de liberação de exudatos nas suas extremidades radiculares (FILHO et al., 2004). Dentre as Fabaceae destaca-se a ervilhaca, mucuna cinza, e ervilha pelo considerável acúmulo de N na MS das raízes e menor decomposição em relação às demais espécies da mesma família. Dessa forma, raízes que apresentam baixa taxa de decomposição podem resultar em alto sequestro de C no solo (RASSE et al., 2005; KONG; SIX, 2010) conforme discutido no Artigo 2. Sistemas baseados em Fabaceae (leguminosas) mostraram incremento nos estoques de C e N no solo, o que evidencia o potencial das Fabaceae em recuperar a MOS pelo sequestro de C no solo (AMADO et al., 2001; DIEKOW et al., 2005). Provavelmente, a proporção equilibrada de N e C adicionada no solo através dos resíduos de culturais de espécies de Fabaceae atende as necessidades metabólicas dos microorganismos em C e N, e desta forma evita a decomposição da MOS. Assim, torna-se evidente a necessidade de inclusão de Poaceae e a correta seleção de Fabaceae em sistemas de sucessão e rotação de culturas para uma melhor reciclagem de nutrientes e retenção de C no solo. Espera-se que os resultados gerados no Artigo 1 poderão contribuir para melhor estimar a adição e acúmulo de C no solo, pois abrange uma grande diversidade de espécies de culturas anuais de diferentes famílias.

Os trabalhos de decomposição realizados para avaliar os efeitos de aditividade ou não aditividade de misturas de resíduos de parte aérea são na maioria realizados com espécies florestais. Os resultados provenientes de espécies de culturas anuais são contraditórios e limitados a poucas espécies agrícolas. A realização do terceiro estudo (Artigo 3) foi realizado para um melhor entendimento das interações entre a mistura de resíduos da mesma planta com diferentes composições químicas (folha e talo) na mineralização do C e N na superfície do solo. Esse aspecto se justifica que em condições de campo, diferentes partes das plantas são decompostos juntas em uma mistura (HÄTTENSCHWILER et al., 2005). Assim, resíduos com uma diferente composição química podem modificar o processo de decomposição da mistura (BERGLUND et al., 2013).

As misturas individuais das 25 espécies mostraram uma grande amplitude e heterogeneidade na composição química (Artigo 3; Figura 1, Tabela 2) e na mineralização do C e N (Artigo 3; Figura 2). De fato, a heterogeneidade química inicialmente foi criada pela mistura em diferentes proporções de folhas e talos das 25 espécies das cinco famílias. Posteriormente, pelo grau de maturidade no momento da coleta dos resíduos; colheita para culturas principais e floração para plantas de cobertura de solo. Os resíduos de Fabaceae apresentaram alto N e componentes solúveis e com grandes diferenças entre folhas e talos. Esses resíduos provocaram efeito não aditivo (antagônico ou negativo) na mineralização do N (Artigo 3; Figura 4b; Figura 6a). Os efeitos sinérgicos (positivo) representaram em média + 9% da mineralização do C. Esses resultados confirmaram nossas hipóteses que plantas leguminosas devido à habilidade de fixar N_2 atmosférico podem produzir resíduos de alta qualidade (principalmente folhas) que podem promover a preservação do N mineral, especialmente em solos pobres em N.

Finalmente, os resultados do Artigo 3 permitem concluir que as interações das misturas são controladas pelo grau de heterogenidade das misturas e disponibilidade de N. No entanto, para algumas espécies de plantas não foi possível estabelecer uma clara correlacão entre as interações e os parâmetros de qualidade dos resíduos (composição química). Esses resultados e de outros estudos recentes evidenciam que as interações relatadas à decomposição em misturas (aditivos ou não aditivos) podem ser controladas por numerosos e complexos mecanismos. A quantificação dos tipos de interações em misturas de resíduos pode já ter sido representados pelos mecanismos já estudados, mas é um grande desafio um melhor entendimento nos estudos futuros.

6 PERSPECTIVAS DE ESTUDOS FUTUROS

A partir da realização, obtenção e análise dos principais resultados do presente trabalho, foi possível identificar alguns aspectos que merecem ser priorizados em estudos futuros relacionados à decomposição de resíduos de culturas anuais usadas em sistemas agrícolas. Alguns trabalhos já foram iniciados e seria importante serem concluídos:

1) Enriquecer através de pulsos semanais com isótopo de C (^{13}C) resíduos de culturas anuais de primavera/verão e outono/inverno em condições de campo.
2) Realizar experimentos de decomposição em condições de campo com resíduos de culturas anuais marcados com ^{13}C e caracterizados pela composição química buscando identificar a contribuição da parte aérea, e principalmente, das raízes para a adição e acúmulo de C no solo. Assim, será possível avançar no conhecimento sobre a relação entre a composição química de resíduos culturais da parte aérea e raízes x decomposição x MOS em condições de campo em sistema de plantio direto.
3) Realizar estudos em condições de campo e de laboratório para avaliar o efeito da disponibilidade de N no solo na mineralização do C e do N de resíduos de raízes de culturas anuais.
4) Realizar estudos em condições de campo e laboratório para avaliar os efeitos de interação que ocorre durante a mineralização do C e N entre resíduos da parte aérea e raízes de culturas anuais no solo.
5) Realizar estudos com maior grau de detalhamento com alguns componentes químicos de resíduos de culturas anuais. Por exemplo, realizar a análise do tipo de polifenóis, hemiceluloses e ligninas.
6) Usar a modelização como ferramenta de apoio na previsão da dinâmica de mineralização do C e do N de resíduos de culturas anuais em sistema de plantio direto, ou seja, da parte aérea mantidos na superfície e das raízes incorporadas ao solo.

7 REFERÊNCIAS BIBLIOGRÁFICAS

AMADO, T.J.C. et al. Potencial de culturas de cobertura em acumular carbono e nitrogênio no solo no plantio direto e a melhoria da qualidade ambiental. **Revista Brasileira de Ciência do Solo**, v. 25, p. 189-197, 2001.

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

CHAPMAN, K.; WHITTAKER, J.B.; HEAL, O.W. Metabolic and faunal activity in litters of tree mixtures compared with pure stands. **Agriculture, Ecosystems & Environment,** v. 24, n. 1-3, p. 33-40, 1988.

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

HADAS, A. et al. Rates of decomposition of plant residues and available nitrogen in soil, related to residue composition through simulation of carbon and nitrogen turnover. **Soil Biology and Biochemistry**, v. 36, n. 2, p. 255-266, 2004.

HOOKER, T.D.; STARK, J.M. Soil C and N cycling in three semiarid vegetation types: response to an in situ pulse of plant detritus. **Soil Biology and Biochemistry**, v. 40, n. 10, p. 2678-2685, 2008.

JARECKI, M.K.; LAL, R. Crop management for soil carbon sequestration. **Critical Reviews in Plant Sciences**, v. 22, n. 6, p. 471-502, 2003.

LAL, R. Soil carbon sequestration to mitigate climate change. **Geoderma**, v. 123, n. 1-2, p. 1-22, 2004.

LOVATO, T. et al. Adição de carbono e nitrogênio e sua relação com os estoques no solo e com o rendimento do milho em sistemas de manejo. **Revista Brasileira de Ciência do Solo,** v. 28, p. 175-187, 2004.

SALAMANCA, E.F.; KANEKO, N.; KATAGIRI, S. Effects of leaf litter mixtures on the decomposition of *Quercus serrata* and *Pinus densiflora* using field and laboratory microcosm methods. **Ecological Engineering,** v. 10, p. 53-73, 1998.

8 ANEXOS

ANEXO A – Instruções referentes aos estilos, normas de citações, referências bibliográficas, tabelas e figuras conforme as normas da revista Plant and Soil.

Citation

Cite references in the text by name and year in parentheses. Some examples:

- Negotiation research spans many disciplines (Thompson 1990).
- This result was later contradicted by Becker and Seligman (1996).
- This effect has been widely studied (Abbott 1991; Barakat et al. 1995; Kelso and Smith 1998; Medvec et al. 1999).

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list. Reference list entries should be alphabetized by the last names of the first author of each work.

- Journal article:

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. doi: 10.1007/s00421-008-0955-8

Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted: Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

- Article by DOI:

Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. doi:10.1007/s001090000086

- Book:

South J, Blass B (2001) The future of modern genomics. Blackwell, London

- Book chapter:

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

- Online document:

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

- Dissertation:

Trent JW (1975) Experimental acute renal failure. Dissertation, University of California

Tables

- All tables are to be numbered using Arabic numerals.
- Tables should always be cited in text in consecutive numerical order.
- For each table, please supply a table caption (title) explaining the components of the table.
- Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.
- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Figures

- To add lettering, it is best to use Helvetica or Arial (sans serif fonts).
- Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).
- Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.
- Avoid effects such as shading, outline letters, etc.
- Do not include titles or captions within your illustrations.
- All figures are to be numbered using Arabic numerals.
- Figure parts should be denoted by lowercase letters (a, b, c, etc.).

ANEXO B – Instruções referentes aos estilos, normas de citações, referências bibliográficas, tabelas e figuras conforme as normas da revista Soil Biology and Biochemistry.

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Web references

As a minimum, the full URL should be given and the date when the reference as last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

References in a special issue

Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Reference style

Text: All citations in the text should refer to:

1. *Single author:* the author's name (without initials, unless there is ambiguity) and the year of publication;

2. *Two authors:* both authors' names and the year of publication;

3. *Three or more authors:* first author's name followed by 'et al.' and the year of publication. Citations may be made directly (or parenthetically). Groups of references should be listed first alphabetically, then chronologically.

Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999). Kramer et al. (2010) have recently shown ....'

Reference list

References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.

- Reference to a journal publication:

Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59.

- Reference to a book:

Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.

- Reference to a chapter in an edited book:

Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith, R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

Tables

Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article.

Figures

Ensure that each illustration has a caption. Supply captions separately, not attached to the figure. A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

9 APÊNDICES

APÊNDICE A – Determinação de polifenóis solúveis totais.

(Modificado de KING; HEALTH, 1967; TIAN et al., 1995)

Reagentes

Folin-Denis
Metanol (CH_3OH)
Carbonato de sódio (Na_2CO_3)
Ácido tânico ($C_76H_52O_46$)

Soluções

Metanol 50%

- Diluir metanol com água na proporção de 1:1

Carbonato de sódio 17%

- Dissolva em balão volumétrico 170g de carbonato de sódio em cerca de 800 ml de água destilada. Posteriormente completar o balão a 1000 ml e misturar bem.

Ácido tânico padrão (0,1 mg/ml)

- Pesar 0,0110g de ácido tânico em um becker de 50 ml (0,1 mg/ml = 0,010g/100 ml, mas isso é somente 90,8% de pureza, então pesar 0,0110g para ser 100%. Transferir o ácido tânico do Becker para um balão volumétrico de 100 ml e misturar bem.

Procedimento analítico

1- Pesar 0,75g de amostra (anotar o peso) e colocar em frasco *snap cap* de 110 ml.
2- Adicione 20 ml de metanol 50%.
3- Tampar os frascos e colocar em banho maria à 80ºC por 1 hora.
4- Filtrar a amostra em papel filtro tipo W*hatman* nº2 (110 mm de diâmetro) em um balão volumétrico de 50 ml. Utilizar funil para suporte do filtro.
5- Lavar o resíduo do s*nap cap* com metanol e continue filtrando com metanol 50% até atingir aproximadamente 40 ml do balão.
6- Completar com metanol até 50 ml e misturar bem a solução do balão.
7- Adicionar cerca de 20 ml de água destilada em um balão volumétrico de 50 ml.
8- Pipetar 1 ml do extrato para o balão.
9- Adicionar 2,5 ml de reagente Folin-Denis.
10- Adicionar 10 ml de carbonato de sódio 17%.
11- Completar a 50 ml com água destilada.
12- Aguarde 30 minutos e leia em absorbância de 760nm. Incluir um extrato em branco com apenas 20 ml de metanol 50%.

Padrões

- Pipetar 0, 1, 2, 3, 4, 5 e 6 ml ou mais (as amostras devem estar dentro da curva) de ácido tânico padrão (0,1 mg /ml) em um frasco volumétrico de 50 ml.
- Adicionar 20 ml de água.
- Adicionar 2,5 ml de reagente Folin-Denis.
- Adicionar 10 ml de carbonato de sódio 17%.
- Completar o volume para 50 ml e misturar bem.

- Aguarde 30 minutos e leia em absorbância de 760nm. Usar o padrão em branco para ajustar o aparelho.

Cálculos

- Plotar em gráfico as mg de ácido tânico (eixo x) e absorbância (eixo y).
- Determinar a equivalência de ácido tânico das amostras e em branco.
- A percentagem total de polifenóis é calculada através da fórmula:

$$\%\, POL = \frac{(TAE * Fc * Fd)}{(10 * peso\ amostra)}$$

TAE = Total equivalente àcido tânico
Fc = Valor inclinação da reta
Fd = Diluições

- O resultado é expresso em % da massa seca à 65ºC

Observações

- O ideal é utilizar água ultra pura (*milli Q*) em todo o processo.
- Caso não for possível realizar a leitura com 1 ml de extrato (etapa 8) será necessário concentrar a amostra. Então, pipetar 2, 3, 4, 5 ml ou mais de extrato.

Referências

KING, H.G.; HEALTH, G.W. The chemical analyses of small samples of leaf material and the relationship between the disappearance and composition of leaves. **Pedobiologia**, v. 7 p. 192-197, 1967.

TIAN, G.; BRUSSAARD, L.; KANG, B.T. An index for assessing the quality of plant residues and evaluating their effects on soil and crop in the subhumid tropics. **Applied Soil Ecology**, v. 2, p. 25-32, 1995.

APÊNDICE B – Determinação de carbono e nitrogênio solúveis em água.

Reagentes

Dicromato de potássio ($K_2Cr_2O_7$)
Ácido sulfúrico (H_2SO_4)
Sulfato ferroso ($FeSO_4.7H_2O$)
Fenantrolina ($C_{12}H_8N_2$)

Soluções

Dicromato de potássio (1,25 molc L^{-1})

- Dissolver 61,28g de $K_2Cr_2O_7$ em balão volumétrico e completar a 1 litro. Adotar procedimento descrito em Tedesco et al. (1995).

Sulfato ferroso (0,5 molc L^{-1})

- Dissolver em balão volumétrico 139g de $FeSO_4$ $7H_2O$ em 400 ml de água destilada. Adicionar 15 ml de H_2SO_4 concentrado e completar a 1 litro. Adotar procedimento descrito em Tedesco et al. (1995).

Procedimento analítico - C solúvel em água

1- Pesar 0,5g de tecido seco a 65ºC moído ou picado (depende do objetivo).
2- Colocar o tecido em *snap cap* de 110 ml e adicionar 60 ml de H_20 destilada.
3- Agitar a mistura em agitador horizontal por 30 minutos com os frascos deitados.
4- Após a agitação, filtrar o material em filtro *Whatman* nº5 (110 mm de diâmetro).
5- Fazer no mínimo 3 brancos passando somente água destilada pelo filtro.
6- Retirar do filtrado 10 ml (amostra pouco concentrada) ou 5 ml (amostra mais concentrada) e colocar em tubo de digestão.
7- Adicionar 5 ml de $K_2Cr_2O_7$ (1,25 molc L^{-1}).
8- Adicionar 10 ml de H_2SO_4 18 M.
9- Realizar digestão a 150º por 30'. Esperar esfriar o material.
10- Fazer 6 brancos (somente $K_2Cr_2O_7$ e H_2SO_4), 3 digeridos junto com as amostras (aquecidos) e 3 deixados em temperatura ambiente (não aquecidos).
11- Titular com $FeSO_4.7H_2O$ (0,5 molc L^{-1}) 0,5 N. A titulação pode ser feita diretamente no tubo de digestão. Usar 3 gotas de indicador ferroin.

Procedimento analítico - N total solúvel em água

1- Retirar do filtrado 5 ml e colocar em tubo de digestão. Fazer no mínimo 3 brancos e 3 padrões. Usar 1 ml de solução padrão 0,7 mg N ml^{-1}.
2- Digerir as amostras, brancos e padrões conforme metodologia adotada para tecido vegetal com todos os reagentes e tempos (Tedesco et al. 1995).
3- Proceder à destilação e titulação com H_2SO_4 0,01 N.

Observação: A análise de N mineral solúvel é feita com 10 ml do filtrado (completar o tubo para 20 ml) e adição simultânea de MgO e Liga de Devarda. Usar 1 ml de padrão 0,05 mg N ml^{-1}. Proceder à titulação com H_2SO_4 0,00125 N.

Referências

TEDESCO, M.J. et al. **Análises de solo, plantas e outros materiais.** Porto Alegre: Universidade Federal do Rio Grande do Sul, Departamento de Solos, 1995. 174p. (Boletim Técnico, 5).

APÊNDICE C – Determinação das frações de Van Soest (soluble, hemicelulose, celulose e lignina).

(Modificado de VAN SOEST, 1963; VAN SOEST et al., 1991)

Reagentes

Sulfato láurico de sódio ($NaC_{12}H_{25}SO_4$)
Etileno diaminote tetracetato dissódico (EDTA)
Borato de sódio decahidratado ($Na_2B_4O_710H_2O$)
Fosfato ácido de sódio anidro ($Na_2HPO_4^-$)
Trietilenoglicol ($C_6H_{14}O_4$)
Brometo cetil trimetil amônio (CTAB)
Ácido sulfúrico (H_2SO_4)
Acetona

Soluções

Solução Detergente Neutra (SDN) – Para fazer 1 litro de solução

a) Pesar 18,61g de EDTA e 6,81g de borato de sódio decahidratado e colocar em um becker com aproximadamente 400 ml de água destilada e aquecer até dissolver com agitador magnético.
b) Fazer outra solução com 30g de sulfato láurico de sódio e 10 ml de trietilenoglicol sem aquecimento e juntar com a primeira solução (a).
c) Pesar 4,56g de fosfato ácido de sódio anidro e dissolver em água destilada por aquecimento, e posteriormente, misturar as demais soluções (a e b). Atentar para não ultrapassar 1 litro para todas as soluções preparadas.
d) A SDN deve ter pH final entre 6,9 a 7,1 (conferir e ajustar).

Solução Detergente ácida (SDA) – Para fazer 1 litro de solução

a) Pesar 20g de CTAB e dissolver em 1 litro de ácido sulfúrico 1 N, previamente padronizado e agitar bem.
b) A solução de H_2SO_4 1 N é feita com a adição 27,48 ml de H_2SO_4 concentrado (96-98% de pureza) em 1 litro de água destilada.

Ácido sulfúrico 72% p/p, 24 N, gravidade específica 1,634

a) Dissolver 667 ml de ácido sulfúrico 96-98% em 333 ml de água destilada em recipiente resistente (becker de plástico). Utilizar gelo em torno do Becker para resfriar a solução. Realizar todo o procedimento na capela.
b) Exemplos práticos:

1 M = 2 N
Então: 24 N = 12 M

Ex. 1: C1 x V1 = C2 x V2
18 M x V1 = 12 M x 12000 (12 litros)
V1 = 8 litros de H_2SO_4 em 4 litros de água destilada

Ex. 2: C1 x V1 = C2 x V2
18 M x V1 = 12 M x 6000 (6 litros)
V1 = 4 litros de H_2SO_4 em 2 litros de água destilada

Procedimento analítico – Determinação da fibra em detergente neutro (FDN)

1) Pesar 0,3g de amostra moída (1 mm) e colocar em tubos de digestão. Anotar o peso.
2) Adicionar 30 ml de SDN.
3) Iniciar a fervura em bloco digestor subindo lentamente a temperatura (50 – 100 – 150ºC) para evitar a espuma. A partir do início da fervura deixar em refluxo por 60 minutos. Cuidar para que a amostra entre em contato com o reagente (ela forma um "sobrenadante" e poderá sair do tubo).
4) Após os 60 minutos de fervura filtrar por sucção o vácuo em cadinho filtrante, previamente pesado e identificado. Deve ser feita imediatamente após a digestão do material, ou seja, quando estiver ainda quente (80-90ºC).
5) Limpar bem o interior do tubo de digestão com água quente (usar pisseta).
6) Lavar bem (2-3 vezes) a fibra dentro do cadinho com água destilada quente (aproximadamente 90ºC).
7) Lavar 2 vezes a fibra com acetona (30-40 ml).
8) Secar a fibra no interior dos cadinhos a 105ºC durante 8 horas ou uma noite. Transferir para dessecador e posteriormente pesar.
9) Queimar os cadinhos com a fibra em mufla a 550ºC por 3 horas. Cuidado com identificação das amostras (fazer croqui). Deixar espaço entre os cadinhos dentro da estufa. Transferir para dessecador os cadinhos quando esta estiver abaixo de 250ºC e pesar em temperatura ambiente.
10) A percentagem de FDN (corrigida de cinzas) é calculada pela diferença entre peso inicial e peso final da amostra.
11) A FRAÇÃO SOLÚVEL é obtida subtraindo de 100 à porcentagem encontrada para FDN.

$$FRAÇÃO\ SOLÚVEL\ (\%) = 100 - \%FDN$$

Observação: Na determinação da FND é separada a parte insolúvel: Celulose, lignina, minerais insolúveis (sílica) e HEMICELULOSE. Solúveis: proteínas, amido, açúcares, ácidos orgânicos e lipídios.

Procedimento analítico – Determinação da fibra em detergente acido (FDA)

1) Pesar 0,6g de amostra moída (1 mm) em tubo de digestão. Anotar o peso.
2) Adicionar 60 ml de SDA.
3) Iniciar a fervura em bloco digestor subindo lentamente a temperatura (50 – 100 – 150ºC) para evitar a espuma. A partir do início da fervura deixar em refluxo por 60 minutos. Cuidar para que a amostra entre em contato com o reagente (ela forma um "sobrenadante" e poderá sair do tubo).
4) Após os 60 minutos de fervura filtrar por sucção por vácuo em cadinho filtrante, previamente pesado e identificado. Deve ser feita imediatamente após a digestão do material, ou seja, quando estiver ainda quente (80-90ºC).
5) Limpar bem o interior do tubo de digestão com água quente (usar pisseta).
6) Lavar bem (2-3 vezes) a fibra dentro do cadinho com água destilada quente (aproximadamente 90ºC).
7) Lavar 2 vezes a fibra com acetona (30-40 ml).

8) Secar a fibra no interior dos cadinhos a 105ºC durante 8 horas ou uma noite. Transferir para dessecador e posteriormente pesar.

Observação: Na determinação da FAD é separada a parte insolúvel: celulose, lignina, minerais insolúveis (sílica) e as solúveis: proteínas, amido, açúcares, ácidos orgânicos, lipídios e HEMICELULOSE.

$$HEMICELULOSE\ (\%) = \%FDN - \%FDA$$

Procedimento analítico – Determinação da lignina (método do ácido sulfúrico)

1) Colocar os cadinhos filtrantes com a fibra proveniente da digestão ácida em uma bandeja plástica com 2 cm de água.
2) Adicionar aproximadamente 30 ml de H_2SO_4 72% em cada cadinho.
3) Misturar a fibra do cadinho com um bastão de vidro fino. Mexer até quebrar todos os grumos e permitir que o ácido entre em contato com toda a amostra.
4) Repetir os passos 2 e 3 por 3 horas.
5) Filtrar com bomba de vácuo. Lavar bem os cadinhos com os resíduos, fora e dentro, com água quente (80-90ºC) até a retirada total do ácido.
6) Secar a fibra no interior dos cadinhos a 105ºC durante 8 horas ou uma noite. Transferir para dessecador e posteriormente pesar.
7) Queimar os cadinhos com a fibra em mufla a 500ºC por 3 horas. Cuidado com identificação das amostras (fazer croqui). Deixar espaço entre os cadinhos dentro da estufa. Transferir para dessecador os cadinhos quando esta estiver abaixo de 250ºC e pesar em temperatura ambiente.

   LIGNINA: A percentagem de lignina é calculada pela diferença de peso da amostra antes e após a queima em mufla.

   CELULOSE: A percentagem de celulose é calculada pela diferença de peso da amostra antes e após e o processo de eliminação da celulose por H_2SO_4 72%.

   Observações:
   - Utilizar balança de precisão em todas as etapas.
   - O somatório da percentagem de fração solúvel, hemicelulose, celulose e lignina do resíduo deve ser 100%. Caso contrário, é necessário usar um fator de correção para ajustar os valores obtidos.
   - Utilizar sempre as mesmas condições para pesar os materiais.

Referências

VAN SOEST, P.J. Use of detergent in the analysis of fibrous feeds. Preparation of fiber residues of low nitrogen content. **Journal Association of Official Analytical Chemists**, v. 46, n. 5, p. 825-835, 1963.

VAN SOEST, P.J; ROBERTSON, J.B.; LEWIS, B.A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. **Journal of Dairy Science**, v. 74, n. 10, p. 3583-3597, 1991.

VITA

Marciel Redin, filho de Enrique Piovesan Redin e Isaura Dalcin Redin, nasceu em Nova Palma, Rio Grande do Sul, Brasil.

Cursou da 1ª a 4ª série na Escola Estadual Dom Érico Ferrari, em Linha Base e da 5ª a 8ª série na Escola Estadual de 1º e 2º Graus Tiradentes em Nova Palma, RS.

Em 1999 ingressou no Exército Brasileiro no Núcleo de Preparação de Oficiais da Reserva (NPOR) do 7º Batalhão de Infantaria Blindada (7ºBIB), na cidade de Santa Maria, RS. No mesmo ano foi declarado Aspirante Oficial R2 do Exército Brasileiro.

Em 2001 chegou à Universidade Federal de Santa Maria (UFSM). Em 2002 iniciou o Curso de Agronomia da UFSM, trabalhou como bolsista de iniciação científica nos Departamentos de Fitotecnia e Solos. Formou-se Engenheiro Agrônomo em 2007.

Em 2008 iniciou o curso de Mestrado no Programa de Pós-graduação em Ciência do Solo da UFSM, sob a orientação do professor Sandro José Giacomini. Recebeu em 2010 o título de mestre em Ciência do Solo.

Em 2010 iniciou Doutorado no Programa de Pós-graduação em Ciência do Solo da UFSM, sob a orientação do professor Sandro José Giacomini. Realizou parte do Doutorado pelo Programa Ciência sem Fronteiras do CNPq no "*Institut National de La Recherche Agronomique"* (INRA) na Unidade "*Environment & Agronomy Division"* de Reims, França. Recebeu em 2014 o título de Doutor em Ciência do Solo.

Entre 2010 a 2012, paralelo ao Doutorado, realizou o Programa Especial de Graduação de Formação de Professores para a Educação Profissional - PEG/UFSM.

Marciel Redin é casado com Luciana Dapieve Patias e moram em Santa Maria, RS, Brasil.