



# Carbon isotope discrimination during litter decomposition can be explained by selective use of substrate with differing $\delta^{13}\text{C}$

Jérôme J. Ngao, Maria-Francesca M.-F. Cotrufo

## ► To cite this version:

Jérôme J. Ngao, Maria-Francesca M.-F. Cotrufo. Carbon isotope discrimination during litter decomposition can be explained by selective use of substrate with differing  $\delta^{13}\text{C}$ . *Biogeosciences Discussions*, 2011, 8 (1), pp.51-82. 10.5194/bgd-8-51-2011 . hal-00964495

**HAL Id: hal-00964495**

**<https://hal.science/hal-00964495>**

Submitted on 28 May 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

This discussion paper is/has been under review for the journal Biogeosciences (BG).  
Please refer to the corresponding final paper in BG if available.

# Carbon isotope discrimination during litter decomposition can be explained by selective use of substrate with differing $\delta^{13}\text{C}$

J. Ngao<sup>1,2,3</sup> and M. F. Cotrufo<sup>1,4</sup>

<sup>1</sup>Dipartimento di Scienze Ambientali, Seconda Università di Napoli, Via Vivaldi 43, 81100 Caserta, Italy

<sup>2</sup>UMR 7618 Biogéochimie et Ecologie des Milieux Continentaux (Bioemco), 46, rue d'Ulm, 75230 Paris CEDEX, France

<sup>3</sup>UMR 547 INRA-UBP PIAF, Centre INRA Clermont-Theix, 234 avenue du Brezet, 63000 Clermont-Ferrand, France

<sup>4</sup>Dept. of Soil and Crop Science, Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, Colorado, USA

Received: 30 November 2010 – Accepted: 16 December 2010 – Published: 4 January 2011

Correspondence to: J. Ngao (jerome.ngao@clermont.inra.fr)

Published by Copernicus Publications on behalf of the European Geosciences Union.

**BGD**

8, 51–82, 2011

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



## Abstract

Temporal dynamics of C isotopic composition ( $\delta^{13}\text{C}$ ) of  $\text{CO}_2$  and leaf litter was monitored during a litter decomposition experiment using *Arbutus unedo* L., as a slow decomposing model substrate. This allowed us (1) to quantify isotopic discrimination variation during litter decomposition, and (2) to test whether selective substrate use or kinetic fractionation could explain the observed isotopic discrimination. Total cumulative  $\text{CO}_2$ -C loss ( $C_L$ ) comprised 27% of initial litter C. Temporal evolution of  $C_L$  was simulated following a three-C-pool model. Isotopic composition of respired  $\text{CO}_2$  ( $\delta_{\text{RL}}$ ) was higher with respect to that of the bulk litter. The isotopic discrimination  $\Delta_{(\text{L/R})}$  varied from  $-2\text{‰}$  to  $0\text{‰}$  and it is mostly attributed to the variations of  $\delta_{\text{RL}}$ . A three-pool model, with the three pools differing in their  $\delta^{13}\text{C}$ , described well the dynamic of  $\Delta_{(\text{L/R})}$ , in the intermediate stage of the process. This suggests that the observed isotopic discrimination between respired  $\text{CO}_2$  and bulk litter is in good agreement with the hypothesis of successive consumption of C compounds differing in  $\delta^{13}\text{C}$  during decomposition. However, to explain also  $^{13}\text{C}$ - $\text{CO}_2$  dynamics at the beginning and end of the incubation the model had to be modified, with discrimination factors ranging from  $-1\text{‰}$  to  $-4.6\text{‰}$  attributed to the labile and the recalcitrance pool, respectively. We propose that this discrimination is also the result of further selective use of specific substrates within the two pools, likely being both the labile and recalcitrant pool of composite nature. In fact, the  $2\text{‰}$   $^{13}\text{C}$  enrichment of the  $\alpha$ -cellulose observed by the end of the experiment, and potentially attributable to kinetic fractionation, could not explain the measured  $\Delta_{(\text{L/R})}$  dynamics.

## 1 Introduction

Soils contain the largest terrestrial carbon (C) pool, and understanding the mechanisms controlling the flux of C is crucial to assess climate change and the potential C sink strength of soils (Jenkinson, 1991). In this context, stable C isotopes are very

**BGD**

8, 51–82, 2011

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



useful metabolic tracers to: (1) identify pathways and quantify rates of litter–C pools into different soil organic matter (SOM) pools (Balesdent et al., 1987; Bernoux et al., 1998; Hobbie et al., 2004; Rubino et al., 2007, 2010), (2) quantify and partition soil CO<sub>2</sub> efflux (Cheng, 1996; Wolfram et al., 2000; Ngao et al., 2005; Subke et al., 2006), and (3) link dissolved organic C (DOC) to respired CO<sub>2</sub> (Bengtson and Bengtsson, 2007). All these studies apply a linear mixing ratio between two (or more) sources and derive the contribution of two (or more) end-members to the pool or flux of interest using mass balance equations (Balesdent et al., 1987; Cheng, 1996). The mass balance approach generally relies on the assumption that there is no <sup>13</sup>C versus <sup>12</sup>C discrimination during heterotrophic respiration of organic matter (OM) substrates (Cheng, 1996; Ekblad et al., 2002; Ngao et al., 2005; Rubino et al., 2007). However, evidences exist that this assumption may be incorrect (Mary et al., 1992; Blagodatskaya et al., 2010). Uncertainties in the stable C isotopic composition ( $\delta^{13}\text{C}$ ) of the C sources may significantly influence the estimates of the relative contribution of end members in mixing models (Philips and Gregg, 2001). Thus, understanding and quantifying isotopic discrimination during heterotrophic respiration and OM decomposition is necessary for accurately estimating belowground C input and CO<sub>2</sub> losses using isotopic methods.

Different theoretical models describing isotopic discrimination during decomposition of litter and SOM have been proposed (Ågren et al., 1996; Feng, 2002; Poage and Feng, 2004). Yet, we lack a full understanding and quantitative modeling of isotopic discrimination during heterotrophic respiration of the kind we have for photosynthesis (Farquhar et al., 1982). That is mainly because in the natural environment heterotrophic respiration arises from the breakdown of a composite substrate (e.g. plant tissue, soil organic matter), made of compounds (e.g. soluble sugars, lipids, structural carbohydrates, etc.) differing in their isotopic composition (Gleixner et al., 1993).

Two relevant processes may participate to differentiate the isotopic composition of the respired CO<sub>2</sub> from that of the OM substrate, during heterotrophic respiration: (i) selective use of C compounds with different  $\delta^{13}\text{C}$ , and (ii) kinetic fractionation, which results from enzymatic discrimination of <sup>13</sup>C versus <sup>12</sup>C (or viceversa) during the

**BGD**

8, 51–82, 2011

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



respiratory process. In the case of selective use, we would observe a difference in  $\delta^{13}\text{C}$  between the product and the substrate, and this would not result from a true isotopic fractionation process during decomposition. Additionally, during soil respiration, also physical fractionation takes place in association to the diffusion of  $\text{CO}_2$  between soil pore and the location where soil  $\text{CO}_2$  efflux is measured, i.e. above the litter layer. In the soil, the latter has been well investigated and theoretical and experimental estimates are available (Amundson et al., 1998; Davidson, 1995; Kayler et al., 2010), thus we do not discuss physical fractionation in this study. We refer to isotopic discrimination ( $\Delta$ ) as to the difference in  $\delta^{13}\text{C}$  between respired  $\text{CO}_2$  (the product) and the OM which it is derived from (the substrate).

To identify whether a kinetic fractionation occurs during heterotrophic respiration, experiments have been conducted on individual substrates. Mary et al. (1992) pioneered this field demonstrating kinetic fractionation in heterotrophic respiration, but a mechanism for this observation is still not clear, and beyond the scope of the present study. Regardless of the type of discrimination (kinetic or selective use) during heterotrophic respiration, the quantification and mechanistic understanding of isotopic discrimination is crucial for the accurate use of natural abundance isotope technique for belowground C cycling work (Werth and Kuzyakov, 2010).

Several studies showed significant isotopic discrimination during microbial consumption of single compounds (Blair et al., 1985) and plant residues (Melillo et al., 1989; Schweizer et al., 1999; Connin et al., 2001; Kristiansen et al., 2004), but the results were highly variable, depending on the substrate and the organisms (bacteria, fungi) investigated. Only few studies looked at changes in  $\delta^{13}\text{C}$  of both respired  $\text{CO}_2$  and complex decomposing substrates (i.e. plant tissues) over time (Fernandez et al., 2003; Osono et al., 2008). And all of them suggest the selective use of C substrate as the main process driving isotopic discrimination during SOM decomposition, but do not present a model to explain those dynamics. Fernandez et al. (2003) suggested that plant quality may play a role in the observed C isotopic dynamics during early stages of litter decomposition, stages during which the lignin fraction have a limited influence

**BGD**

8, 51–82, 2011

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



on both mass loss and  $\delta^{13}\text{C}$  of early-decomposing litter (Ngao et al., 2005; Osono et al., 2008).

The present study aims at: (1) characterizing the temporal dynamics of  $\delta^{13}\text{C}$  of the  $\text{CO}_2$  and of the leaf litter substrate from which it is respired, (2) quantifying isotopic discrimination ( $\Delta$ ) during litter decomposition and eventual variation of  $\Delta$  over time, and (3) testing whether selective substrate use or kinetic fractionation can explain eventually observed isotopic discrimination. We hypothesized that (i) the  $\delta^{13}\text{C}$  of respired  $\text{CO}_2$  varies with time during litter decomposition and that, at any given time, it is different from the  $\delta^{13}\text{C}$  of the bulk litter; (ii) at the study time scale, the variation in  $\delta^{13}\text{C}$  of respired  $\text{CO}_2$  is mainly explained by a selective use of C sources of different  $\delta^{13}\text{C}$  and, thus, that (iii) no significant kinetic fractionation for a given C substrate, is expected. We performed a laboratory litter decomposition experiment using *Arbutus unedo* L. leaf litter, as a model composite substrate with a relatively low decay rates (Cotrufo et al., 2010b). A set of litter samples was incubated to follow the temporal evolution of respired  $\text{CO}_2$  and its  $\delta^{13}\text{C}$ , while another larger set was incubated to characterize temporal evolution of chemical and isotopic composition of the remaining litter, by destructive samplings. We used cellulose as a model substrate to experimentally test the kinetic fractionation hypothesis. Our hypotheses were farther explored by model simulations.

## 2 Material and methods

### 2.1 Field litter sampling

The leaf litter was collected in a 50-ha coppiced stand dominated by the evergreen shrub *Arbutus unedo* (L.) at Tolfa-Allumiere (42°11'30" N, 11°55'30" E, 180 m a.s.l., Central Italy). For a full site description see Cotrufo et al. (2010a). At the site, *A. unedo* leaves fall in two main periods: May–June and October–November. Fully senesced *A. unedo* leaf litter was collected from standing trees in a strict *A. unedo*-dominated area

**BGD**

8, 51–82, 2011

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



in late May 2006, returned to the lab and litter was left to air dry. All leaves gathered together were assumed to represent a homogenous new fresh litter pool, as they were mixed well for several days during air drying.

## 2.2 Experimental setup

5 *A. unedo* litter samples were incubated in air tight mason jars filled each with 3–5 g of air-dried litter. A sub-set of air-dried litter was oven-dried at 70 °C in order to correct for the residual moisture. Before incubation, litter was sprinkled with deionized water to adjust moisture at 135% in gravimetric water content by weighting the jar. Airtight jars were incubated in the dark at 25 °C. During the entire incubation period, litter water  
10 content was adjusted gravimetrically and headspace flushed. The first set of samples (Set 1,  $n=10$ ) was dedicated to periodical CO<sub>2</sub> efflux and  $\delta^{13}\text{C}$  of respired CO<sub>2</sub> measurements (see below). A second set (Set 2,  $n=70$ ) was periodically harvested ( $n=5$  at each harvest) for mass loss and chemical and isotopic analyses (see below).

## 2.3 CO<sub>2</sub> measurements

15 The incubation period (i.e. between two measurement dates) varied from 1 day at the beginning of the experiment to around 60 days by the end of it. The length of each incubation period was established in order to avoid excessive CO<sub>2</sub> enrichment and O<sub>2</sub> depletion in the jar headspace and to minimize the number of samplings. For each of the 10 replicate jars of Set 1, at the end of each incubation period, CO<sub>2</sub> concentration  
20 within the jar headspace was estimated by diluting a small amount of jar headspace air into a closed loop, which comprised an infrared gas analyzer (IRGA) Li-840 (Licor, Lincoln, USA) connected to a 0.5-L glass buffer, used to increase the total system volume (0.87 L). Before injection, the IRGA was left to run at a flow rate of 1 L min<sup>-1</sup> in closed circuit, and a three-way connector placed at the IRGA outlet was left opened to  
25 the exit in order to reach both CO<sub>2</sub> concentration and pressure equilibrium (both were real-time monitored). Then, 0.02 L ( $V_{\text{Syr}}$ ) of headspace air was sampled with a syringe

**BGD**

8, 51–82, 2011

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



and injected into the circuit. The syringe was removed and the three-way connector was closed, allowing pressure equilibration without any CO<sub>2</sub> concentration perturbation. The CO<sub>2</sub> concentration within the jar headspace ( $C_J$ , in ppmv) was calculated as:

$$C_J = V_{\text{Syst}}(C_{\text{Final}} - C_{\text{Ini}})V_{\text{Syr}}^{-1} \quad (1)$$

where  $V_{\text{Syst}}$  is the volume of the entire closed circuit (i.e. 0.87 L),  $C_{\text{Ini}}$  is the CO<sub>2</sub> concentration within the system before syringe injection (volume  $V_{\text{Syr}}$ ), and  $C_{\text{Final}}$  is the CO<sub>2</sub> concentration within the system 1 m after syringe injection. Total respired C ( $C_L$ ) is calculated by extrapolating  $C_J$  to the jar volume (1.37 L), assuming a molar volume of 24.79 L mol<sup>-1</sup> and expressed on a litter dry mass basis (mg C g<sub>DM</sub><sup>-1</sup> d<sup>-1</sup>).

Then after, the CO<sub>2</sub> accumulated during the incubation period within the jar headspace was cryogenically trapped. For this purpose, the jar was connected and opened to a cryogenic purification line (described by Bertolini et al., 2005). The headspace air was driven into the line at a constant flow rate of 0.1 L min<sup>-1</sup> (mass flow controller Dwyer GFC 2104, Michigan City, USA) by a high vacuum pump (Mini-Task Varian Inc., Palo Alto, USA) placed at the end of the line. Along the line, first loop was bathed in an ethanol-dry ice mixture for scrubbing water vapor, and a second loop bathed in liquid nitrogen trapped CO<sub>2</sub> after setting the internal pressure at 250 mbar. Trapped CO<sub>2</sub> was transferred into a 6-mm PIREX<sup>®</sup> glass tube filled with reduced copper flakes. Cryogenic purification duration varied according to the CO<sub>2</sub> concentration within the jar determined as previously described. Afterwards, the jars were left opened for 30 min for CO<sub>2</sub> and O<sub>2</sub> equilibration as well as for stabilization of microbial activity. At this point, the instantaneous litter respiration rate was determined by measuring the increase rate of the CO<sub>2</sub> concentration within the jar for five minutes using the Li-840 IRGA in closed mode. This was done in order to estimate the length of the subsequent incubation period. All remaining background CO<sub>2</sub> within the jar headspace air was scrubbed for five minutes; so that exclusively litter respired CO<sub>2</sub> would accumulate during the following incubation period.

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion





## 2.4 Litter harvest, and chemical analyses

At each CO<sub>2</sub> measurement date, five replicate jars were harvested. Litter was oven-dried, weighed for mass loss and ground for chemical analyses. On the initial litter and on the litter samples collected at the end of the incubation experiment,  $\alpha$ -cellulose was extracted from 0.2 g of ground litter enclosed in a porous bag (Filter bags, Ankom Technology, Macedon, USA) with two successive extractions using 5% NaOH solution for 2 h at 60 °C (Loader et al., 1997). The remaining material was washed 3 times with boiling distilled water, removing most of soluble compounds, fatty materials, resins, tannins and hemicelluloses. Then lignin was removed by a 36 h extraction in a 7% sodium chlorite (NaClO<sub>2</sub>) solution adjusted to a pH of 4–5 by adding 4–5 mL of 96% acetic acid (Loader et al., 1997). The remaining  $\alpha$ -cellulose that was washed with boiling distilled water, oven-dried at 50 °C, weighted and prepared for isotopic analyses. Carbon concentration (g C g<sub>DM</sub><sup>-1</sup>) of bulk litter and  $\alpha$ -cellulose fraction were measured by an elemental analyzer (Flash EA 1112 NC, CE Instrument, Wigan, UK). All analyses were performed singularly on the five litter replicates.

## 2.5 Isotopic analysis

The  $\delta^{13}\text{C}$  of the bulk litter ( $\delta_{\text{BL}}$ ) and the  $\alpha$ -cellulose fraction ( $\delta_{\text{cell}}$ ) was measured by an elemental analyzer (Flash EA 1112 NC, CE Instrument, Wigan, UK) connected to an Isotope Ratio Mass Spectrometer (IRMS, Delta Plus, Thermo-Finnigan, Bremen, Germany). The calibration of the EA-IRMS setup was performed by analyzing the C6 ( $\delta^{13}\text{C} = -10.80 \pm 0.47\text{‰}$ ; C content=42.13%) and C8 ( $\delta^{13}\text{C} = -18.3\text{‰} \pm 0.29\text{‰}$ ; C content=26.67%) standard material provided by the International Atomic Energy Agency (IAEA, Vienna, Austria), as well as with an internal standard ( $\delta^{13}\text{C} = -27.37 \pm 0.16\text{‰}$ ; C content=8.93  $\pm$  0.89%).

To eliminate N<sub>2</sub>O, all CO<sub>2</sub> samples, enclosed in PIREX® tubes containing several grams of copper were preliminary baked at 400 °C during 30 min in order to reduce eventual N<sub>2</sub>O into N<sub>2</sub> and CuO. This pre-treatment avoids overlapping in mass

**BGD**

8, 51–82, 2011

### Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



44 (confounding  $\text{N}_2\text{O}$  with  $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ ) and mass 45 (due to  $\text{O}^{17}$ ) measurements. The  $\delta^{13}\text{C}$  of each  $\text{CO}_2$  sample ( $\delta_{\text{RL}}$ ) was determined by IRMS (Delta Plus, Thermo-Finnigan, Bremen, Germany) in Dual Inlet mode. The  $\text{CO}_2$  samples from a first  $\text{CO}_2$ -in-air cylinder ( $\delta^{13}\text{C} = -11.02 \pm 0.05\text{‰}$ , certificated by the Commonwealth Scientific and Industrial Research Organization (CSIRO) Atmospheric Research, Aspendale, Australia) and a second  $\text{CO}_2$ -in-air cylinder ( $\delta^{13}\text{C} = -25\text{‰}$ , Messer Griesheim GmbH, Krefeld, Germany) was cryogenized and analyzed for correcting fractionation occurring during the cryogenization step or instrumental biases. All  $\delta^{13}\text{C}$  values are expressed against the international PDB standard.

## 2.6 Calculations and statistical analysis

All variables are expressed as daily rates calculated from either ten ( $\text{CO}_2$  variables) or five (bulk litter material) replicates. A non-linear regression procedure (NLIN procedure, SAS v8, SAS Institute, USA) was used to fit the total litter  $\text{CO}_2$ -C loss ( $C_L$ , expressed as cumulated C loss relative to initial litter C amount, in  $\text{g}_\text{C} \text{ g}_{\text{ini}}^{-1}$ ) over time ( $t$ ) to a multi-pool single exponential model according to:

$$C_L(t) = \sum_i f_i (1 - e^{-k_i t}) + \varepsilon \quad (2)$$

where  $f_i$  is the fraction of a C pool  $i$  participating to  $C_L$  and  $k_i$  is the decomposition rate constant of the respective C pool ( $\text{d}^{-1}$ ), and  $\varepsilon$  a residual term. We compared the Eq. (2) for either  $i=2$  (two-pool model) or  $i=3$  (three-pool model) by calculating for each model the Akaike Information Criterion, corrected for small samples ( $\text{AIC}_\text{C}$ , McQuarrie and Tsai, 1998):

$$\text{AIC}_\text{C} = \ln(\text{RSS}/(n-p)) + (n+p)/(n-p-2) \quad (3)$$

where  $n$  is the number of samples, RSS is residual sum of square and  $p$  is the number of model parameters. The lowest  $\text{AIC}_\text{C}$  value indicates the best model.

**BGD**

8, 51–82, 2011

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



In order to explore the causes of the variations of  $\delta_{RL}$  over time, we first derived the fractional contributions of each of tree pools ( $f_{C_i}$ ):

$$f_{C_i}(t) = f_i \left( 1 - e^{-k_i t} \right) / C_L(t) \quad (4)$$

with the  $f_i$  and  $k_i$  parameters and  $C_L$  values from the Eq. (2). Then, a mass balance approach was adopted in order to test if the  $\delta^{13}C$  of the different pools weighted by their fractional contribution could reproduce the  $\delta^{13}C$  of litter  $CO_2$ -C loss ( $\delta_{RL}$ ) according to:

$$\delta_{RLsim}(t) = \sum_i f_{C_i}(t) \delta_i(t) \text{ and } \sum_i f_{C_i}(t) = 1 \quad (5)$$

where  $\delta_i$  is the  $\delta^{13}C$  the C pool  $i$ . Moreover, Eq. (5) assumes no direct fractionation on the  $i$  pool (i.e.  $\delta_i = \delta^{13}C$  of  $CO_2$  derived from the  $i$  pool).

C isotopic discrimination ( $\Delta_{(L/R)}$ ) between litter OM and respired  $CO_2$  was calculated as:

$$\Delta_{(L/R)} = (\delta_{BL} - \delta_{RL}) / (1 + \delta_{RL}) \quad (6)$$

where  $\delta^{13}C_{BL}$  is the  $\delta^{13}C$  of the remaining litter material. Linear regression analyses were performed to calculate the  $CO_2$  concentration rate ( $ppmv s^{-1}$ ), hereafter converted in litter respiration rate (in  $\mu mol g_{DM}^{-1} s^{-1}$ ). The  $\delta^{13}C$  values of the different C components (respired  $CO_2$ , bulk litter,  $\alpha$ -cellulose) were averaged per sampling day. One-way ANOVAs analyses were used to compare daily  $\delta^{13}C$  mean values among the different C components by date (STATGRAPHICS Plus 4.1).

## 3 Results

### 3.1 Litter respired $CO_2$

Litter respiration rate was high (around  $2 mg C g_{DM}^{-1} d^{-1}$ ) at the beginning of the incubation, reduced by half in the following two months to decline slower until the 4th

**BGD**

8, 51–82, 2011

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



month (day of incubation DOI 137), where it remained stable until the end of the experiment (Fig. 1). Very little variation was observed between replicate litters (standard error ( $n=10$ ) not exceeding  $0.0002 \text{ mg C g}_{\text{DM}}^{-1} \text{ d}^{-1}$ ). The total cumulated  $C_L$  was  $457 \pm 53 \text{ mg C y}^{-1}$ , corresponding to around 27% of initial litter C. Temporal evolution of  $C_L$  (Fig. 2) was modeled following Eq. (2) ( $R^2 > 0.999$ ;  $\text{rmse} = 0.003$ ;  $p < 0.0001$ ) using three C pools ( $i=3$ ). Adding a third pool enhanced significantly the description of dynamics of  $\text{CO}_2$ -C loss, as compared to a two-pool model ( $i=2$ ;  $R^2 = 0.998$ ;  $\text{rmse} = 0.005$ ;  $p = 0.0001$ ), with the  $\text{AIC}_C$  value ( $\text{AIC}_C = -9.918$ ) being lower for the three- than for the two-pool model ( $\text{AIC}_C = -8.271$ ). The three C pools contributed at different proportions to the total litter respired  $\text{CO}_2$ , and had significantly different decomposition rate constants  $k$  (Table 1). The fractional contribution of each C pool, calculated according to Eq. (4) varied with time. The labile pool was the main contributor during the first 70 days while after the intermediate pool became the main contributor to litter  $\text{CO}_2$ -C loss (Fig. 2).

## 3.2 Remaining litter

Mean litter mass-C loss averaged 27% of initial mass-C over one year. Mass-C loss and  $\text{CO}_2$ -C loss were very close in daily averages ( $R^2 = 0.95$ ,  $p < 0.001$ , Fig. 3), allowing us to assume that (1) C mass loss exclusively occurred by microbial  $\text{CO}_2$  production as opposed to either fragmentation or leaching, and (2) the two sets decomposed in a similar way. The extracted  $\alpha$ -cellulose concentration ranged from  $13.80 \pm 0.02\%$  to  $5.72 \pm 1.37\%$  of total dry matter (Fig. 4).

## 3.3 Carbon isotopes

### 3.3.1 Temporal dynamics

The  $\delta^{13}\text{C}$  of litter respired  $\text{CO}_2$  ( $\delta_{\text{RL}}$ ) increased significantly during the first 3 days of incubation by about  $1\text{‰}$ , and decreased to  $-29.36 \pm 0.38\text{‰}$  at the end of incubation.

**BGD**

8, 51–82, 2011

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



In contrast, the  $\delta^{13}\text{C}$  of remaining bulk litter ( $\delta_{\text{BL}}$ ) did not vary significantly throughout the experiment, averaging  $-29.14 \pm 0.01\text{‰}$  (Fig. 5). Thus, the C isotopic discrimination  $\Delta_{(\text{L/R})}$  ranged from  $-2\text{‰}$  to  $0\text{‰}$  (Fig. 5), and it is attributable to the variations of  $\delta_{\text{RL}}$ . The  $\delta_{\text{RL}}$  values were positively correlated to litter  $\alpha$ -cellulose concentration values (Fig. 6). The  $\delta^{13}\text{C}$  of  $\alpha$ -cellulose ( $\delta_{\text{cell}}$ ) averaged  $-28.81 \pm 0.25\text{‰}$  in initial litter samples, while it averaged  $-26.74 \pm 0.16\text{‰}$  in the most advanced decomposing litter samples, highlighting a significant  $^{13}\text{C}$  enrichment of  $\alpha$ -cellulose during decomposition ( $p=0.001$ , Fig. 5) by around  $2\text{‰}$ .

### 3.3.2 Model simulations

To test for the selective use of substrate hypothesis, we constrained the mass balance Eq. (5) by assuming no kinetic discrimination on any of the three pools, with  $\delta_1$  being equal to  $-29.09\text{‰}$ , the measured  $\delta^{13}\text{C}$  of the remaining bulk litter at the end of the incubation period – the recalcitrant pool,  $\delta_2$  being equal to  $-25.5\text{‰}$ , as a mean  $\delta^{13}\text{C}$  of soluble compounds – the labile pool – (Schweizer et al., 1999), and  $\delta_3$  as the initial  $\delta^{13}\text{C}$  of  $\alpha$ -cellulose (i.e.  $-28.81\text{‰}$ ). As result of this simulation, the computed  $\delta_{\text{RL}}$  ( $\delta_{\text{RLsim1}}$ ) were in very good agreement for a period between DOY 66 and 227, during which the intermediate pool is the major contributor to total litter  $\text{CO}_2$ -C loss (Fig. 7). This result suggested that the observed  $^{13}\text{CO}_2$  dynamics can be explained by a three-pool decay model with the three pools having different C isotope ratios, and that the  $\alpha$ -cellulose could be used as a proxy for the intermediate pool. However, it fails to explain the  $^{13}\text{CO}_2$  dynamics at the beginning (DOY 0–65) and end (DOY 228–365) of the incubation period (Fig. 7), suggesting either that kinetic discrimination may be occurring, or that the labile and recalcitrant pools are composite, in terms of the  $\delta^{13}\text{C}$  of the specific compounds that contribute to those pools.

To explore if kinetic fractionation could explain the discrepancy between  $\delta_{\text{RL}}$  and  $\delta_{\text{RLsim1}}$ , the measured change in  $\delta^{13}\text{C}$  of  $\alpha$ -cellulose was incorporated to the Eq. (5), assuming a linear interpolation between the two mean values (i.e. constant kinetic

**BGD**

8, 51–82, 2011

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



fractionation), with the other  $\delta_i$  values (recalcitrant and labile) being the same as above. Taking into account the measured cellulose  $^{13}\text{C}$ -enrichment actually led to a larger discrepancy between the computed  $\delta_{\text{RL}}$  ( $\delta_{\text{RLsim2}}$ ) and the measured  $\delta_{\text{LR}}$  (Fig. 7).

As an alternative test, we modified the  $\delta$  values of the labile and recalcitrant pool by introducing a discrimination factor ( $a_i$ ) into the Eq. (5), which lead to the following equation:

$$\delta_{\text{RLsim3}} = f_{\text{C1}}(\delta_1 + a_1) + f_{\text{C2}}(\delta_2 + a_2) + f_{\text{C3}}(\delta_{\text{cell}}) \quad (7)$$

with the  $\delta_i$  values being the same as above. We used Eq. (7) to compute  $\delta_{\text{RL}}$  ( $\delta_{\text{RLsim3}}$ , Fig. 7). Between DOY 1 and 31, the  $\delta_{\text{RLsim3}}$  was found to be sensitive to the  $a_2$  (i.e. of the labile pool), while between DOY 308 and 371, the model was sensitive to  $a_1$  (i.e. of the recalcitrant pool). We found that  $\delta_{\text{RLsim3}}$  best matched  $\delta_{\text{RL}}$ , when  $a_1$  was set at  $-1\text{‰}$  between DOY 1 and 4 and at  $-0.5\text{‰}$  between DOY 8 and 31, and when  $a_2$  was set of at  $-4.6\text{‰}$  between DOY 308 and 371, while in all other period both  $a_1$  and  $a_2$  were set equal to 0 (Fig. 7).

## 4 Discussion

Isotopic discrimination during  $\text{CO}_2$  production has been observed in several studies involving consumption of composite substrate such as plant tissues (Schleser et al., 1999; Schweizer et al., 1999; Fernandez et al., 2003; Kristiansen et al., 2004), soil organic C (Šanctručková et al., 2000) or SOM (Crow et al., 2006; Blagodatskaya et al., 2010). In two of these studies and despite the difference in level of complexity of the involved substrates, the  $\delta^{13}\text{C}$  of respired  $\text{CO}_2$  was initially depleted with respect to initial bulk material and evolved with time through more  $^{13}\text{C}$ -enriched  $\text{CO}_2$  (Fernandez et al., 2003; Crow et al., 2006). In our study, we found the opposite temporal evolution, as shown by the  $\Delta_{(\text{L/R})}$  varying from  $-2\text{‰}$  to  $0\text{‰}$ . This contradictory result may be partly explained by the high lignin content of *A. unedo* leaf litter (35.85%, Piermatteo, 2007), as well as being a tannin-rich species (Rogosic et al., 2006). These secondary

**BGD**

8, 51–82, 2011

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



compounds are known to be generally strongly  $^{13}\text{C}$ -depleted towards water soluble carbohydrates of the same origin (Gleixner et al., 1993) consumed during the early stages of decomposition (Ngao et al., 2009). Also this would explain the rather  $^{13}\text{C}$ -depleted signal of our initial bulk litter ( $-29.05\text{‰}$ ) compared to respired  $\text{CO}_2$  (Fig. 5) or labile C compounds such as root and stem phloem sucrose (which are enriched by about 5‰ in *A. unedo* trees from the same site, data not shown).

Most of the  $\delta^{13}\text{C}$ -based studies have attributed the origin of isotopic discrimination of soil  $\text{CO}_2$  efflux or during litter decomposition to either a selective use of C substrates differing in  $\delta^{13}\text{C}$  or to kinetic fractionation during microbial breakdown of specific compounds. But, to date, no consensus has been reached as, for example, shown by the contradictory conclusions of Boström et al. (2007) and Rubino et al. (2007). Different studies found evidences in support of the selective use of substrate as the mechanism of isotopic discrimination during litter decomposition. Fernandez et al. (2003) and Crow (2006) showed significant correlations between the initial respired  $\text{CO}_2$  (during which  $\Delta$  is negative) and the labile C content of composite residues. Recently, Blagodatskaya et al. (2010) conducted soil incubations with C3- and C4-planted soil, and they concluded that the preferential use of different substrates drove isotopic discrimination among SOM, microbial C and respired  $\text{CO}_2$ , microbial fractionation being negligible.

In our study, we showed that during decomposition of  $\delta_{\text{RL}}$  varied over time and that this variation can be partly explained by a three pool model (i.e. a labile, an intermediate stable and a recalcitrant pool), where the pools differentially contribute to litter decomposition and differ in their  $\delta^{13}\text{C}$ . Moreover, the  $\delta_{\text{BL}}$  did not significantly vary, likely because the relatively small total C loss (maximum of 27% of initial), coupled with a relatively small variation in the  $\delta^{13}\text{C}$  of the three pools (within 3.5‰) did not make the changes in  $\delta^{13}\text{C}$  of the remaining C appreciable. Thus, the  $\Delta_{(\text{L/R})}$  varied due to changes in  $\delta^{13}\text{C}_{\text{RL}}$ . In addition, it was found that  $\delta_{\text{RL}}$  was significantly correlated to the concentration of  $\alpha$ -cellulose, which represented around 15% of the total initial *A. unedo* litter dry mass, indicating that the cellulose C fraction is a major driver of the temporal evolution of  $\delta_{\text{RL}}$ .

**BGD**

8, 51–82, 2011

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion





Temporal evolution of litter respired  $\text{CO}_2$  was best described using a three-pool model (Andren and Paustian, 1987). Several authors modeled their cumulated  $\text{CO}_2$  dynamics using a two-pool model (Fernandez et al., 2003; Rubino et al., 2007), but in our case, adding a third pool improved significantly the explicative efficiency of the model. The  $C_L$  evolution revealed three kinetic C pools with significantly different  $k$  values (in the order of those found by Couteaux et al. (1998)). The three-pool model was used to derive the fractional contribution of each pool, allowing building an isotopic mass balance for testing to which extent the preferential use of C pools drove the  $\delta_{\text{RL}}$  dynamics (Eq. 4; Fig. 7), assuming that the three pools were unique with distinct and constant  $\delta^{13}\text{C}$  values. This approach revealed that the observed isotopic discrimination could be explained only during the intermediate phase of decomposition, when the intermediate pool makes the largest fractional contribution to litter respiration (Fig. 2), thus suggesting that the only pool that could be considered unique was indeed the intermediate pool which in our case is well described by the  $\alpha$ -cellulose. In fact, when the  $\delta^{13}\text{C}$  values of the labile and recalcitrant pools were allowed to change (Eq. 7) the simulated  $\delta_{\text{RLsim3}}$  better matched the measured values, suggesting that either some kinetic fractionation was occurring or that those two pools were not unique, and that within them preferential use of substrates with different  $\delta^{13}\text{C}$  was taking place. Further experimental work needs to be done to clarify the above results. We offer here our interpretation based on data and speculation.

(1) The labile pool is likely a composite pool, made of dead microbial biomass C, sugars, organic acids and other soluble organic compounds, easily consumed by microbes (Cadish and Giller, 1997; Ngao et al., 2009). We originally assumed that the  $\delta^{13}\text{C}$  of the labile pool ( $\delta_2$ ) is unique and corresponds to that measured for litter soluble compounds by Schweizer et al. (1999). Indeed this assumption may be biased because small  $\delta^{13}\text{C}$  differences may occur among soluble compounds extracted from different litter and because the chosen  $\delta^{13}\text{C}$  value of the labile pool ( $-25.5\text{‰}$ ) is likely more similar to that of microbial C than of water-soluble C (lower to ca.  $1\text{--}2\text{‰}$ ), as determined in a C3 soil by Pelz et al. (2005) in agreement with Šanctručková et al. (2000),

**BGD**

8, 51–82, 2011

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion





which would contribute to respiration at the very early stage of litter decay. Damesin and Lelarge (2003) measured the  $\delta^{13}\text{C}$  of sugars of newly fallen leaves (i.e. fresh leaf litter) to be around  $-28\text{‰}$ . When we modified our model and assumed, for the initial decay period, a  $\delta^{13}\text{C}$  of the labile fraction  $1\text{‰}$  to  $0.5\text{‰}$  lower than that used successively, the computed  $\delta_{\text{RLsim3}}$  better matched the measured  $\delta_{\text{RL}}$  (Fig. 7). We therefore suggest that the labile fraction is a composite pool – of possibly 3 sub-fractions with slightly different  $\delta^{13}\text{C}$ , and with the relatively most  $^{13}\text{C}$ -depleted compounds being respired earlier in the decomposition process.

(2) We assumed that the  $\delta^{13}\text{C}$  of the intermediate pool is a unique pool which corresponds to the  $\delta^{13}\text{C}$  measured for  $\alpha$ -cellulose on the initial litter sample. This assumption allowed to successfully simulating the  $\delta_{\text{RL}}$  for the period during which the intermediate pool is the main contributor to  $C_L$ . Moreover, this assumption was also supported by the similarity in the relative size of the modeled intermediate pool ( $f_3=16.80\%$ , Table 1) and that of the  $\alpha$ -cellulose in the initial litter ( $13.80\%$ , Fig. 4). The observed  $^{13}\text{C}$ -enrichment of the  $\alpha$ -cellulose during decomposition does not explain the observed  $\Delta_{(\text{L/R})}$  dynamics and, thus, we suggest that kinetic fractionation even if it may occur (see below) is not the main mechanism behind isotopic discrimination during litter decomposition. As mentioned previously, further work has to be done to better describing this change of  $\delta^{13}\text{C}$  to better integrate it within our model

(3) We initially assumed that the recalcitrant C pool is a unique fraction and that its  $\delta^{13}\text{C}$  corresponds to the  $\delta^{13}\text{C}$  of the remaining litter. However, as for the labile pool, introducing for the recalcitrant pool a discrimination factor in Eq. (7) of  $-4.6\text{‰}$  between DOY 308 and 371, allowed to successfully simulating the  $\delta_{\text{RL}}$  ( $\delta_{\text{RLsim3}}$ , Fig. 7). This discrimination factor is consistent with Schweizer et al. (1999) that reported lignin values which are  $^{13}\text{C}$ -depleted by  $4.6\text{‰}$  with respect to cellulose in C3 leaves (see also the references in Hobbie and Werner (2004) for lignin values in plant materials). In light of those findings, we suggest that also the recalcitrant pool is a composite pool, with the more  $^{13}\text{C}$ -depleted lignin component contributing the majority of this pool at the latest stage of decomposition.

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Our results suggested that temporal  $\delta^{13}\text{C}$ -CO<sub>2</sub> dynamics can be explained by the selective use of different C pools with different  $\delta^{13}\text{C}$  varying over time. But it also highlighted that while three pools are enough to describe leaf litter decay rates, more substrates with distinct  $\delta^{13}\text{C}$  are needed to describe  $\Delta_{(\text{L/R})}$  dynamics, at least during the initial and late stage of decomposition.

Indeed, we also observed a  $^{13}\text{C}$ -enrichment of  $\alpha$ -cellulose extracted from the decomposing litter over time. This result questioned the validity of assumption that the constant  $\delta^{13}\text{C}$  of the C pools used for the mass balance equations (i.e. Eq. 5), and opens to the possibility of kinetic fractionation during consumption of single substrate. However, as said above, at our experiment time scale, the 2‰-enrichment of  $\alpha$ -cellulose throughout litter decomposition is in the same order of magnitude of the  $^{13}\text{C}$ -enrichment of litter respired CO<sub>2</sub>, and thus it may not explain the observed  $\Delta_{(\text{L/R})}$  dynamics.

Although it is possible that isotopic fractionation occurred during the cellulose extraction, all our samples were processed altogether in the same NaOH and NaClO<sub>2</sub> baths, making it unlikely. Tannin–proteins complexes also can be produced during decomposition (Preston et al., 1997) and influence the  $\delta^{13}\text{C}$  of bulk litter but were removed during the NaOH digestion (Loader et al., 1997). With regards to the kinetic discrimination during microbial respiration of individual C pools, several authors showed an isotopic fractionation against  $^{13}\text{C}$  during microbial respiration of a single C substrate such as glucose, leading to depleted respired CO<sub>2</sub> (Blair et al., 1985; Mary et al., 1992; Šanctručková et al., 2000) or not (Ekblad et al., 2002). Schleser et al. (1999) reported isotopic discrimination during thermal decomposition of wood cellulose. However, to our knowledge, this is the first study that measured the  $\delta^{13}\text{C}$  of a polymerized substrate over time, and observed a variation during litter incubations. Contrary to Engelking et al. (2007), this result suggests that some kinetic fractionation process might have also taken place during the decomposition of cellulose. We can identify three possible ways by which this fractionation had occurred, which do not exclude each other:

**BGD**

8, 51–82, 2011

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



1. A carbon isotopic fractionation of the cellulase complex, which may promote a preferential use of “light” parts of  $\alpha$ -cellulose by microorganisms. This effect could originate from intra-molecular heterogeneity in  $^{13}\text{C}$  within the cellulose, as a consequence of discrimination during photosynthetic and post-photosynthetic processes, and the production of secondary compounds (Hobbie and Werner, 2004). To our knowledge, there is no information on such kinetic fractionation of any parts of this enzymatic complex (endo- and exo-glucanase,  $\beta$ -glucosidase) during the disruption of the crystalline structure followed by depolymerization, as shown for the Krebs cycle (Blair et al., 1985; Geissler et al., 2009).

2. A preferential release of  $^{12}\text{CO}_2$  versus  $^{13}\text{CO}_2$  during cellulose breakdown was already shown by Blair et al. (1985). An isotopic fractionation related to  $\text{CO}_2$  production by the microbial biomass, was also evidenced by Blagodatskaya et al. (2010) but it was very minor with regard to the preferential use effect.

3. An isotopic discrimination due to microbial community shift during cellulose decomposition, of the kind that was shown by Haichar et al. (2007) for bacteria. Moreover, Henn and Chapela (2000) showed that the fungal community structure may induce an isotopic discrimination when trioses are assimilated after extracellular cellulose digestion or not when glucose is directly assimilated.

Clearly the transformations of C compounds during decomposition need to be better described to constrain isotopic-based models, improving the knowledge of microbial isotopic discrimination and further applications in field partitioning of soil  $\text{CO}_2$  efflux.

## 5 Conclusions

Litter respired  $\text{CO}_2$  was  $^{13}\text{C}$ -enriched respective to the bulk litter material. This discrimination was shown to be related to a selective use of C sources of different  $\delta^{13}\text{C}$ . Modeling the temporal variation of litter decomposition through  $\text{CO}_2$ -C loss favored

a time-varying fractionation of (at least) three different pools to the temporal variation of respired CO<sub>2</sub>. Our data were best described by an isotopic mass balance of such model including the parameters of remaining litter material, suggesting that the isotopic discrimination observed between respired CO<sub>2</sub> and bulk litter is mainly driven by selective consumption of various C compounds differing in  $\delta^{13}\text{C}$  and in decay rates. However, while three pools well explain litter decay dynamics, more pools with slightly distinct  $\delta^{13}\text{C}$  appear to be needed to account for  $\Delta_{(L/R)}$  dynamics, in particular at the initial and late stages of litter decomposition. Additionally, our study showed an increase of the  $\delta^{13}\text{C}$  of decomposing litter  $\alpha$ -cellulose, but proved that kinetic fractionation of this pool alone cannot explain the observed  $\Delta_{(L/R)}$  dynamics. These results confirm that isotopic discrimination during decomposition of litter and other composite OM substrates needs to be taken into account to prevent biases in determining soil C dynamics by natural abundance stable C isotopes (Werth and Kuzyakov, 2010), which can be corrected for by using the proposed approach.

**Acknowledgements.** The authors thank warmly M. Rubino (CSIRO, Australia) for the technical implementation of the cryogenic line and the improvements of the IRMS measurements procedure. The authors also thank D. Piermatteo (Second University of Naples, Italy) for providing field litter decomposition data. C. Sirignano (Second University of Naples, Italy) is thanked for her skillful IRMS measurements and maintenance. The authors gratefully acknowledge C. Stewart (Colorado State University, Colorado) for checking for correct use of English language. The IRMS instrument is located at the Center for Isotopic Research for Cultural and Environmental heritage (CIRCE). This work is a part of the “Advanced Laser techniques to Investigate Carbon isotope discrimination during decomposition” (ALICE) project funded by the European Union through the Marie Curie Actions for Transfert of Knowledge (Contract MTK–CT–2004–014532). We also acknowledge the two anonymous reviewers for their expertise and useful comments that helped to improve the present manuscript.

**BGD**

8, 51–82, 2011

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



## References

- Ågren, G. I., Bosatta, E., and Balesdent, J.: Isotope discrimination during decomposition of organic matter: a theoretical analysis, *Soil Sci. Soc. Am. J.*, 60, 1121–1126, 1996.
- Amundson, R., Stern, L., Baisden, T., and Wang, Y.: The isotopic composition of soil and soil-respired CO<sub>2</sub>, *Geoderma*, 82, 83–114, 1998.
- Andren, O. and Paustian, K.: Barley straw decomposition in the field – a comparison of models, *Ecology*, 68, 1190–1200, 1987.
- Balesdent, J., Mariotti, A., and Guillet, B.: Natural C-13 abundance as a tracer for studies of soil organic matter dynamics, *Soil Biol. Biochem.*, 19, 25–30, 1987.
- Bengtson, P. and Bengtsson, G.: Rapid turnover of DOC in temperate forests accounts for increased CO<sub>2</sub> production at elevated temperatures, *Ecol. Lett.*, 10, 783–790, 2007.
- Bernoux, M., Cerri, C. C., Neill, C., and de Moraes, J. F. L.: The use of stable carbon isotopes for estimating soil organic matter turnover rates, *Geoderma*, 82, 43–58, 1998.
- Blair, N., Leu, A., Munoz, E., Olsen, J., Kwong, E., and Marais, D.: Carbon isotopic fractionation in heterotrophic microbial metabolism, *Appl. Environ. Microb.*, 50, 996–1001, 1985.
- Blagodatskaya, E., Yuyukina, T., Blagodatsky, S., and Kuzyakov, Y.: Turnover of soil organic matter and of microbial biomass under C3-C4 vegetation change: consideration of <sup>13</sup>C fractionation and preferential substrate utilization, *Soil Biol. Biochem.*, 43, 159–166, 2010.
- Boström, B., Comstedt, D., and Ekblad, A.: Isotope fractionation and C-13 enrichment in soil profiles during the decomposition of soil organic matter, *Oecologia*, 153, 89–98, 2007.
- Cadish, G. and Giller, K. E.: *Driven By Nature: Plant Litter Quality and Decomposition*, CABI publishing, Oxon, UK, 1997.
- Cheng, W.: Measurement of rhizosphere respiration and organic matter decomposition using natural <sup>13</sup>C, *Plant Soil*, 183, 263–268, 1996.
- Connin, S. L., Feng, X., and Virginia, R. A.: Isotopic discrimination during long-term decomposition in an arid land ecosystem, *Soil Biol. Biochem.*, 33, 41–51, 2001.
- Cotrufo, M. F., Alberti, G., Inglima, I., Marianovich, H., LeCain, D., Zaldei, A., Peressotti, A., and Miglietta, F.: Changes in summer drought intensity affect soil carbon accumulation in Mediterranean woodland, submitted to *Global Change Biol.*, 2010a.
- Cotrufo, M. F., Ngao, J., Marzaioli, F., and Piermatteo, D.: Inter-comparison of methods for quantifying leaf litter decomposition rates, *Plant Soil*, 334, 365–376, doi:10.1007/s11104-010-0388-0, 2010.

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



- Couteaux, M. M., McTiernan, K. B., Berg, B., Szuberla, D., Dardenne, P., and Bottner, P.: Chemical composition and carbon mineralisation potential of Scots pine needles at different stages of decomposition, *Soil Biol. Biochem.*, 30, 583–595, 1998.
- Crow, S. E., Sulzman, E. W., Rugh, W. D., Bowden, R. D., and Lajtha, K.: Isotopic analysis of respired CO<sub>2</sub> during decomposition of separated soil organic matter pools, *Soil Biol. Biochem.*, 38, 3279–3291, 2006.
- Davidson, G. R.: The stable isotopic composition and measurement of carbon in soil CO<sub>2</sub>, *Geochim. Cosmochim. Ac.*, 59, 2485–2489, 1995.
- Damesin, C. and Lelarge, C.: Carbon isotope composition of current-year shoots from *Fagus sylvatica* in relation to growth, respiration and use of reserves, *Plant Cell Environ.*, 26, 207–219, 2003.
- Eklblad, A., Nyberg, G., and Högberg, P.: <sup>13</sup>C-discrimination during microbial respiration of added C3-, C4- and <sup>13</sup>C-labelled sugars to a C3-forest soil, *Oecologia*, 131, 245–249, doi:10.1007/s00442-002-0869-9, 2002.
- Engelking, B., Flessa, H., and Joergensen, R. G.: Microbial use of maize cellulose and sugarcane sucrose monitored by changes in the <sup>13</sup>C/<sup>12</sup>C ratio, *Soil Biol. Biochem.*, 39, 1888–1896, 2007.
- Farquhar, G. D., O’leary, M. H., and Berry, J. A.: On the relationship between carbon isotope discrimination and the inter-cellular carbon-dioxide concentration in leaves, *Aust. J. Plant Physiol.*, 9, 121–137, 1982.
- Feng, X.: A theoretical analysis of carbon isotope evolution of decomposing plant litters and soil organic matter, *Global Biogeochem. Cycles*, 16, 1119, doi:10.1029/2002GB001867, 2002.
- Fernandez, I., Mahieu, N., and Cadisch, G.: Carbon isotopic fractionation during decomposition of plant materials of different quality, *Global Biogeochem. Cy.*, 17, 1075, doi:10.1029/2001GB001834, 2003.
- Gessler, A., Tcherkez, G., Karyanto, O., Keitel, C., Ferrio, J. P., Ghashghaie, J., Kreuzwieser, J., and Farquhar, G. D.: On the metabolic origin of the carbon isotope composition of CO<sub>2</sub> evolved from darkened light-acclimated leaves in *Ricinus communis*, *New Phytol.*, 181, 374–386, doi:10.1111/j.1469-8137.2008.02672.x, 2009.
- Gleixner, G., Danier, H. J., Werner, R. A., and Schmidt, H. L.: Correlations between the C-13 content and secondary plant-products in different cell compartments and that in decomposing basidiomycetes, *Plant Physiol.*, 102, 1287–1290, 1993.
- Haichar, F. Z., Achouak, W., Christen, R., Heulin, T., Marol, C., Marais, M. F., Mougel, C.,

**BGD**

8, 51–82, 2011

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



- Ranjard, L., Balesdent, J., and Berge, O.: Identification of cellulolytic bacteria in soil by stable isotope probing, *Environ. Microbiol.*, 9, 625–634, 2007.
- Henn, M. R. and Chapela, I. H.: Differential C isotope discrimination by fungi during decomposition of C3- and C4-derived sucrose, *Appl. Environ. Microb.*, 66, 4180–4186, 2000.
- 5 Jenkinson, D. S., Adamsand, D. E., and Wild, A.: Model estimates of CO<sub>2</sub> emissions from soil in response to global warming, *Nature*, 351, 304–306, 1991.
- Hobbie, E. A. and Werner, R. A.: Intramolecular, compound-specific, and bulk carbon isotope patterns in C3 and C4 plants: a review and synthesis, *New Phytol.*, 161, 371–385, 2004.
- 10 Hobbie, E. A., Johnson, M. G., Rygielwicz, P. T., Tingey, D. T., and Olszyk, D. M.: Isotopic estimates of new carbon inputs into litter and soils in a four-year climate change experiment with Douglas-fir, *Plant Soil*, 259, 331–343, 2004.
- Kayler, Z. E., Sulzman, E. W., Rugh, W. D., Mix, A. C., and Bond, B. J.: Characterizing the impact of diffusive and advective soil gas transport on the measurement and interpretation of the isotopic signal of soil respiration, *Soil Biol. Biochem.*, 42, 435–444, 2010.
- 15 Kristiansen, S. M., Brandt, M., Hansen, E. M., Magid, J., and Christensen, B. T.: <sup>13</sup>C signature of CO<sub>2</sub> evolved from incubated maize residues and maize-derived sheep faeces, *Soil Biol. Biochem.*, 36, 99–105, 2004.
- Loader, N. J., Robertson, I., Barker, A. C., Switsur, V. R., and Waterhouse, J. S.: An improved technique for the batch processing of small wholewood samples to alpha-cellulose, *Chem. Geol.*, 136, 313–317, 1997.
- 20 Mary, B., Mariotti, A., and Morel, J. L.: Use of C-13 variations at natural abundance for studying the biodegradation of root mucilage, roots and glucose in soil, *Soil Biol. Biochem.*, 24, 1065–1072, 1992.
- Ngao, J., Epron, D., Brechet, C., and Granier, A.: Estimating the contribution of leaf litter decomposition to soil CO<sub>2</sub> efflux in a beech forest using C-13-depleted litter, *Global Change Biol.*, 11, 1768–1776, 2005.
- 25 Ngao, J., Bernhard-Reversat, F., and Loumeto, J. J.: Changes in eucalypt litter quality during the first three months of field decomposition in a Congolese plantation, *Appl. Soil Ecol.*, 42, 191–199, 2009.
- 30 Osono, T., Takeda, H., and Azuma, J.: Carbon isotope dynamics during leaf litter decomposition with reference to lignin fractions, *Ecol. Res.*, 23, 51–55, 2008.
- Pelz, O., Abraham, W. R., Saurer, M., Siegwolf, R., and Zeyer, J.: Microbial assimilation of plant-derived carbon in soil traced by isotope analysis, *Biol. Fert. Soils*, 41, 153–162, 2005.

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion





- Philips, D. L. and Gregg, J. W.: Uncertainty in source partitioning using stable isotopes, *Oecologia*, 127, 171–179, 2001.
- Piermatteo, D.: Modelling C and N cycles in Mediterranean terrestrial ecosystems, Ph.D. thesis, Dep. Of Environ. Sci., Second Univ. of Naples, Italy, 2007.
- 5 Poage, M. A. and Feng, X. H.: A theoretical analysis of steady state delta C-13 profiles of soil organic matter, *Global Biogeochem. Cy.*, 18, 13, doi:10.1029/2003GB002195, 2004.
- Preston, C. M., Trofymow, J. A., Sayer, B. G., and Niu, J. N.: C-13 nuclear magnetic resonance spectroscopy with cross-polarization and magic-angle spinning investigation of the proximate-analysis fractions used to assess litter quality in decomposition studies, *Can. J.*  
 10 *Bot.*, 75, 1601–1613, 1997.
- McQuarrie, A. D. R. and Tsai, C. L.: Regression and Time Series Model Selection, World Scientific, Singapore, 1998.
- Rogosic, R. E., Estell, J., Skobic, D., Martinovic, A., and Maric, S.: Role of species diversity and secondary compound complementarity on diet selection of Mediterranean shrubs by goats,  
 15 *J. Chem. Ecol.*, 32, 1279–1287, doi:10.1007/s10886-006-9084-1, 2006.
- Rubino, M., Bertolini, T., De Angelis, P., D'Onofrio, A., Dungait, J. A. J., Evershed, R. P., Lagomarsino, A., Lubritto, C., Merola, A., Terrasi, F., and Cotrufo, M. F.: Carbon input belowground is the major C flux contributing to leaf litter mass loss: evidences from a <sup>13</sup>C labelled-leaf litter experiment, *Soil Biol. Biochem.*, 42, 1009–1016, 2010.
- 20 Rubino, M., Lubritto, C., D'Onofrio, A., Terrasi, F., Gleixner, G., and Cotrufo, M. F.: An isotopic method for testing the influence of leaf litter quality on carbon fluxes during decomposition, *Oecologia*, 154, 155–166, 2007.
- Šantrůčková, H., Bird, M. I., and Lloyd, J.: Microbial processes and carbon–isotope fractionation in tropical and temperate grassland soils, *Funct. Ecol.*, 14, 108–114, 2000.
- 25 Schleser, G. H., Frielingsdorf, J., and Blair, A.: Carbon isotope behaviour in wood and cellulose during artificial aging, *Chem. Geol.*, 158, 121–130, 1999.
- Schweizer, M., Fear, J., and Cadisch, G.: Isotopic (C-13) fractionation during plant residue decomposition and its implications for soil organic matter studies, *Rapid Commun. Mass Sp.*, 13, 1284–1290, 1999.
- 30 Subke, J. A., Inglisma, I., and Cotrufo, M. F.: Trends and methodological impacts in soil CO<sub>2</sub> efflux partitioning: A meta-analytical review, *Global Change Biol.*, 12, 921–943, 2006.
- Swift, M. J., Heal, O. W., and Anderson, J. M.: Decomposition in Terrestrial Ecosystems, Blackwell Scientific Publications, Oxford, UK, 1979.

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion





- Werth, M. and Kuzyakov, Y.:  $^{13}\text{C}$  fractionation at the root-microorganisms-soil interface: a review and outlook for partitioning studies, *Soil Biol. Biochem.*, 42, 1372–1384, 2010.
- Wolfram, S., Neubert, R., Levin, I., Fischer, N., and Sonntag, C.: Determination of microbial versus root-produced  $\text{CO}_2$  in an agricultural ecosystem by means of  $\delta^{13}\text{C}\text{CO}_2$  measurements in soil air, *Tellus B*, 52, 909–918, 2000.

5

**BGD**

8, 51–82, 2011

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



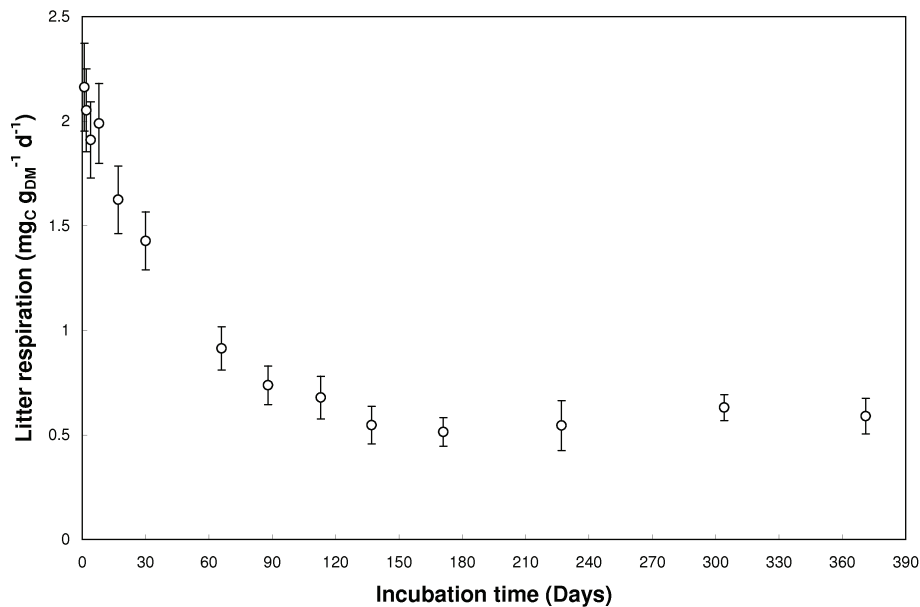
## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

**Table 1.** Values of fractional proportions ( $f_i$ , unitless) and decomposition rate constants ( $k_i$ ,  $\text{d}^{-1}$ ) fitted from the three-C pool model (Eq. 2). For constraining Eq. (4),  $\delta_1$  was set equal to the measured  $\delta^{13}\text{C}$  of remaining bulk litter ( $\delta_{\text{BL}}$ ),  $\delta_2$  was set equal to  $-25.5\text{‰}$  (Schweizer et al., 1999), and  $\delta_3$  was set at  $-28.81\text{‰}$ , i.e. the  $\delta^{13}\text{C}$  of cellulose in initial litter.

C pool	$f_i$	$k_i$	$\delta_i$
1 – Recalcitrant	0.776	0.0002	$\delta_{\text{BL}}$
2 – Labile	0.061	0.0635	$-25.5$
3 – Intermediate	0.163	0.0067	$-28.81$

[Title Page](#)
[Abstract](#)
[Introduction](#)
[Conclusions](#)
[References](#)
[Tables](#)
[Figures](#)
[◀](#)
[▶](#)
[◀](#)
[▶](#)
[Back](#)
[Close](#)
[Full Screen / Esc](#)
[Printer-friendly Version](#)
[Interactive Discussion](#)

**Fig. 1.** Temporal evolution of mean litter respiration rate ( $\pm$  standard errors ,  $n=10$ ).

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

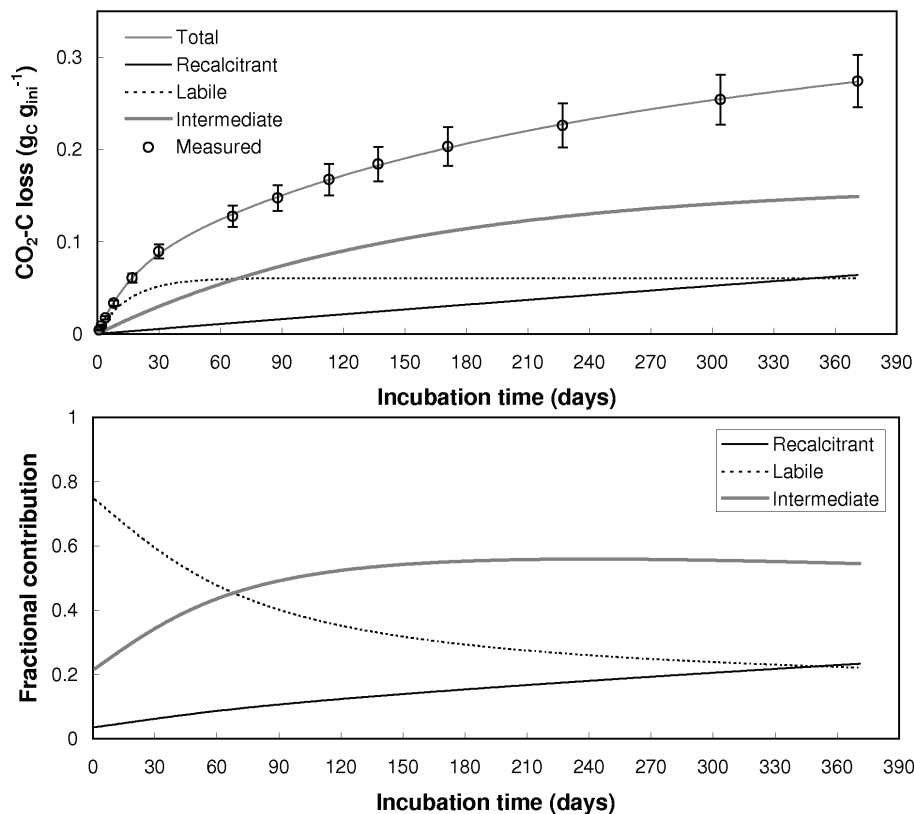
Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion





**Fig. 2.** Upper panel. Temporal evolution of cumulative respired CO<sub>2</sub>-C ( $C_L$  in the text) expressed as fraction of initial litter C (open circle, mean  $\pm$  standard errors). Temporal evolution of total cumulative respired CO<sub>2</sub>-C (solid grey line) and that of the three C pools were simulated according to Eqs. (2) and (4), respectively (see text for details, see Table 1 for model parametrisation). Lower panel. Temporal evolution of fractional contribution (unitless) to total litter cumulated CO<sub>2</sub>-C of each C pool as calculated by Eq. (4).

# Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

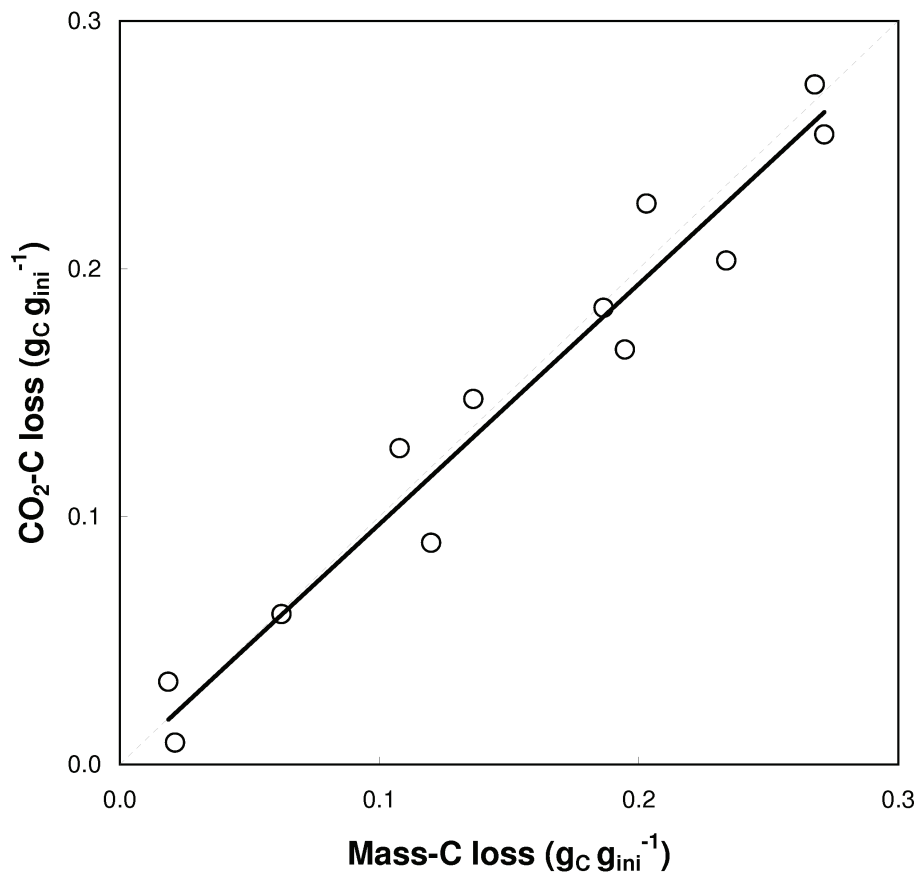
Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

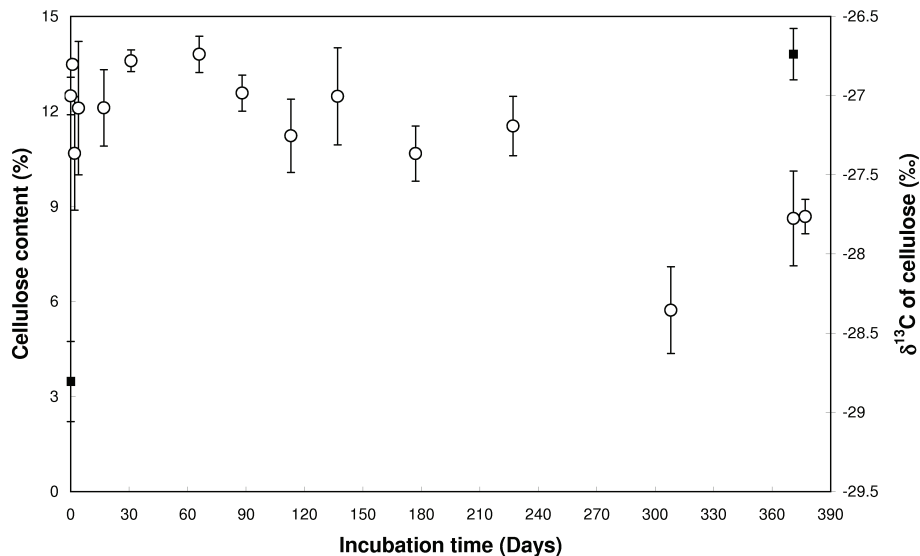




**Fig. 3.** Comparison between cumulated  $\text{CO}_2$ -C loss ( $C_L$  in the text) estimated by  $\text{CO}_2$  concentration measurements and mass-C loss by litter harvests, both expressed as fraction of initial litter C ( $\text{g}_\text{C} \text{g}_{\text{ini}}^{-1}$ ). The  $\text{CO}_2$ -C and mass-C fractions were linearly related such as  $\text{CO}_2$ -C = 0.9693 mass-C ( $R^2 = 0.95$ ,  $p < 0.001$ ).

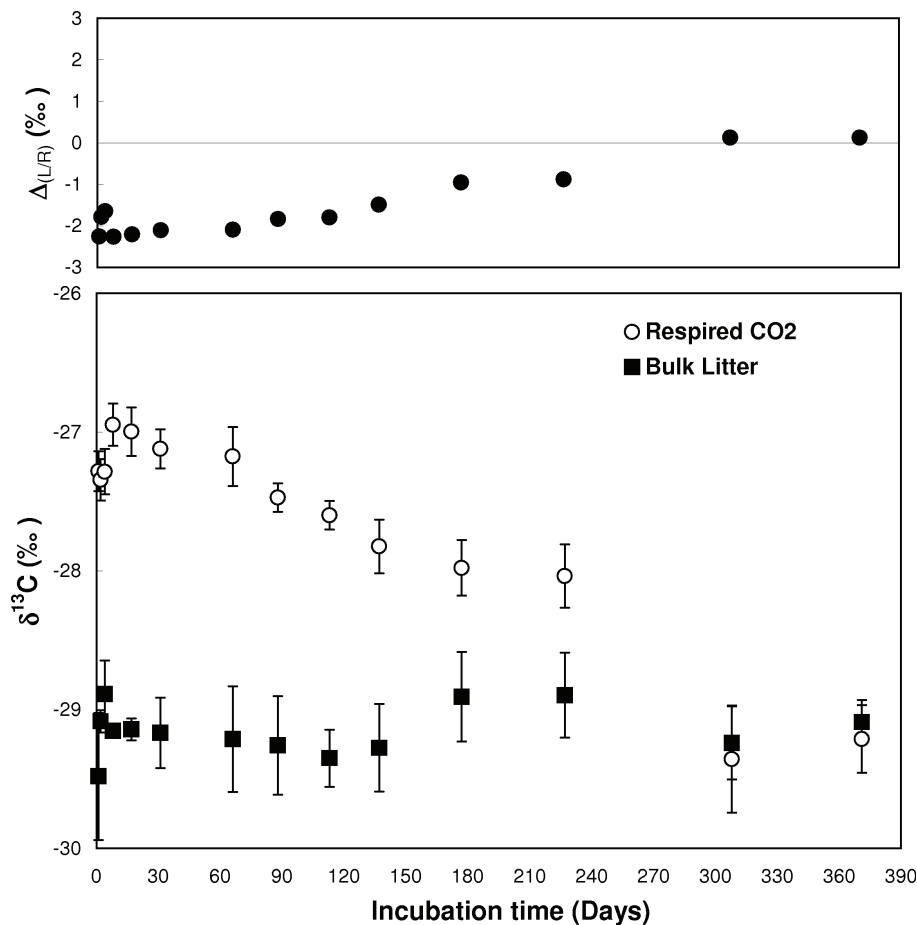
## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

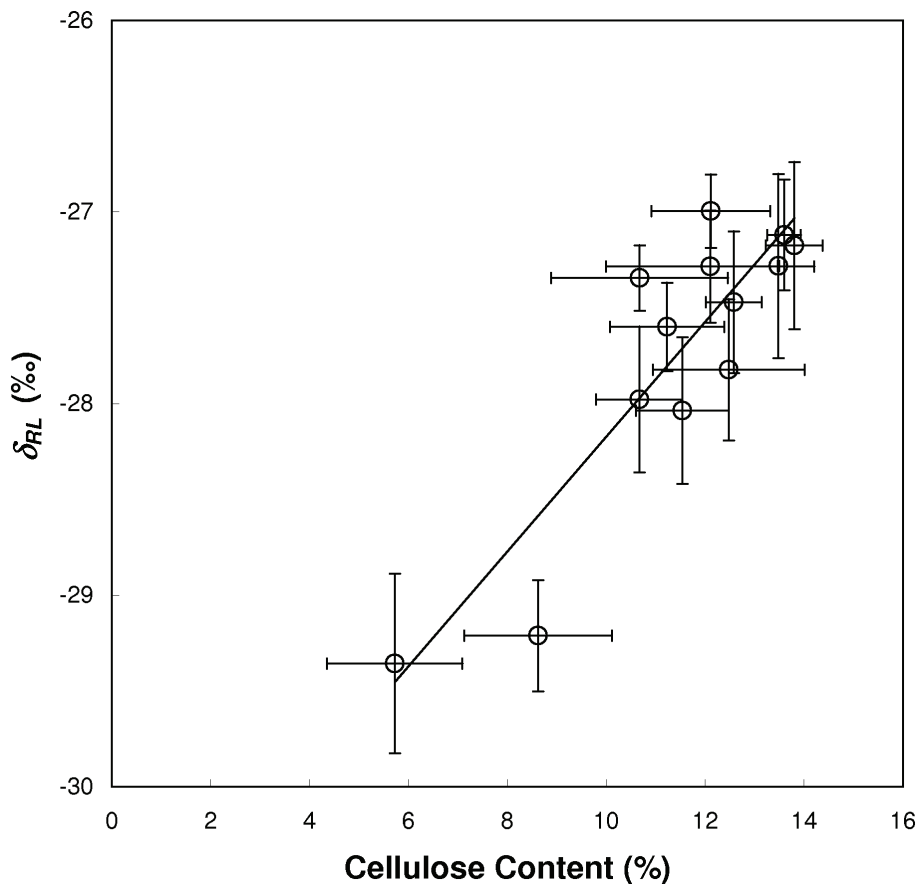


**Fig. 4.** Temporal variation of  $\alpha$ -cellulose content (open circle) and of its isotopic composition ( $\delta^{13}\text{C}$ , closed squares). Error bars are standard errors.

[Title Page](#)
[Abstract](#)
[Introduction](#)
[Conclusions](#)
[References](#)
[Tables](#)
[Figures](#)
[◀](#)
[▶](#)
[◀](#)
[▶](#)
[Back](#)
[Close](#)
[Full Screen / Esc](#)
[Printer-friendly Version](#)
[Interactive Discussion](#)

**Fig. 5.** Lower panel: Temporal evolution of mean  $\delta^{13}\text{C}$  of respired  $\text{CO}_2$  (open circles,  $n=10$ ) and bulk litter organic matter (closed squares,  $n=5$ ). Upper panel: Temporal evolution of isotopic discrimination ( $\Delta_{(L/R)}$ ) between respired  $\text{CO}_2$  and bulk litter. Error bars are standard errors.



**Fig. 6.** Relationship between remaining cellulose content and  $\delta^{13}\text{C}$  of litter respired  $\text{CO}_2$  ( $\delta_{RL}$ ). The error bars are standard errors. The solid line is the linear regression as:  $\delta_{RL} = 0.3 (\text{Cellulose}) - 31.17$  ( $R^2 = 0.78$ ,  $p < 0.05$ ).

## Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

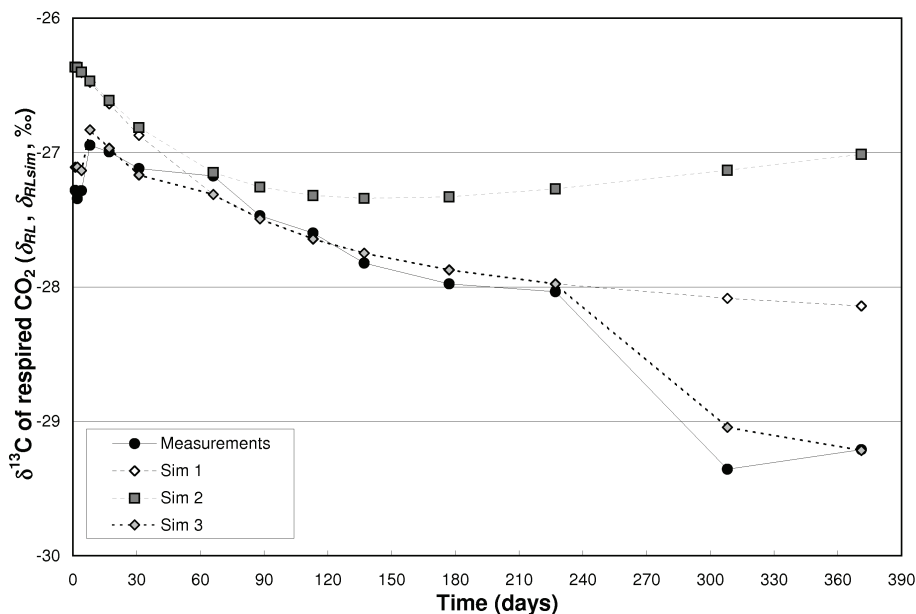
Interactive Discussion





# Carbon isotopic discrimination during litter decomposition

J. Ngao and M. F. Cotrufo



**Fig. 7.** Time-course of the measured (Mes,  $\delta_{\text{RL}}$ , circles) or simulated (Sim,  $\delta_{\text{RLsim}}$ , diamonds)  $\delta^{13}\text{C}$  of litter  $\text{CO}_2\text{-C}$  loss as computed by Eq. (5) (see the text for more details). The simulation Sim1 was performed by applying directly the Eq. (5) with the  $f_{\text{C}_i}$  and  $\delta_i$  parameters as detailed in the text, as well for the simulation Sim2 which takes also into account the change in  $\delta^{13}\text{C}$  of the cellulose used as a proxy of the intermediate pool. The simulation Sim3 was the same as Sim1, plus accounting in Eq. (5) a discrimination factor of  $-1\%$  on the labile pool at  $t=\text{DOY } 1$  ( $a_1$  in Eq. 7), and discrimination factors of  $-4.6\%$  for recalcitrant pools ( $a_3$ , Eq. 7) at  $t=\text{DOY } 371$ .

[Title Page](#)
[Abstract](#)
[Introduction](#)
[Conclusions](#)
[References](#)
[Tables](#)
[Figures](#)
[◀](#)
[▶](#)
[◀](#)
[▶](#)
[Back](#)
[Close](#)
[Full Screen / Esc](#)
[Printer-friendly Version](#)
[Interactive Discussion](#)
