ELSEVIER

Soil Biology & Biochemistry 37 (2005) 1105–1112

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Palatability trials on hardwood leaf litter grown under elevated CO_2: a stable carbon isotope study

M. Francesca Cotrufo[a,*], Bert Drake[b], James R. Ehleringer[c]

[a]*Department of Environmental Sciences, Second University of Naples, Via Vivaldi 43, 81100 Caserta, Italy*
[b]*Smithsonian Environmental Research Centre, P.O. Box 28, Edgwater, MD 21037, USA*
[c]*Department of Biology, University of Utah, 257 S 1400 E, Salt Lake City, UT 84112-0840, USA*

Received 1 April 2004; received in revised form 12 November 2004; accepted 15 November 2004

Abstract

The palatability to isopods and microbes of a broad range of hardwood leaf litter, derived from three field CO_2-enrichment experiments in the USA, was investigated, using $\delta^{13}C$, to trace the C flow from litter to isopods and to CO_2 respired by microbial decomposition. Leaf litter grown under elevated CO_2 had $\delta^{13}C$ values ranging from −39 to −45‰, which were significantly different from ambient litter $\delta^{13}C$ values of around −30‰. Litter palatability to isopods of the *Porcellio* sp. was tested by incubating ambient- and elevated-CO_2 litter, and a mixture of the two, in the presence of isopods for 14 days, under environmentally controlled conditions; $\delta^{13}C$ was measured on litter and isopods' body before and after incubation. In an additional experiment, litter was incubated in the absence of fauna for 30 days, and on five occasions the $\delta^{13}C$ of the CO_2 respired from litter was measured. The ^{13}C label was clearly carried from the litter source to the isopods' bodies, and their faeces. For microbial-respired CO_2, $\delta^{13}C$ was significantly higher than that of the litter source, suggesting preferential degradation of substrates enriched in ^{13}C as compared to those in the overall litter. With the exception of *Quercus myrtifolia* leaf litter, elevated CO_2 did not affect the palatability to isopods nor the microbial degradation of any of the litters, possibly as a result of unaltered litter N concentration. However, significant differences in litter palatability and decay rates were observed among the different species. With this study, the use of isotopically labelled litter material was confirmed as a key methodology that can significantly contribute to the advancement of the understanding of litter decomposition and of the quantification of C fluxes in the process.

Keywords: Stable C isotopes; Elevated CO_2; Leaf litter; *Porcellio* sp.; Decomposition

1. Introduction

Plant tissues generated under elevated atmospheric carbon dioxide ($^{at}CO_2$) are often of a different quality than tissues generated under ambient $^{at}CO_2$. In particular, they can show a decrease in nitrogen (N) concentrations (Cotrufo et al., 1998a); a general, but not statistically significant, increase in lignin concentration (Norby et al., 2001a); a change in the composition and concentration of secondary plant metabolites (Peñuelas et al., 1997); a modification of their physical structure, with thicker leaves (Vu et al., 1989). On the basis of these observations, it has been hypothesised that plant tissues generated under elevated $^{at}CO_2$ would be less palatable to insects (Roth and Lindroth, 1995). This hypothesis, however, can result in two opposite responses: (1) insects would consume a higher quantity of plant tissues generated under elevated $^{at}CO_2$, because of their poorer nutritional value (higher C/N ratio) to satisfy their needs; (2) insects would avoid feeding on the less nutritious tissues, with all the implications and feedbacks that this change may generate at the community level.

In order to investigate if plant tissues generated under elevated $^{at}CO_2$ are less palatable to insects and also to microbial decomposers, and to determine which of the two above responses is more likely to occur, experiments must be designed such that the absolute palatability (i.e. consumption) and preferential use of ambient and elevated $^{at}CO_2$ tissues can be assessed. A powerful technique to study food webs and C flows between resources and feeders is

* Corresponding author. Tel.: +39 823 274647; fax: +39 823 274605.
E-mail address: mfrancesca.cotrufo@unina2.it (M. Francesca Cotrufo).

0038-0717/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2004.11.009

the use of C isotope tracing (Radajewski et al., 2000). The variation in the natural abundance of ^{13}C was used in several studies of faunal assimilation of organic matter (Martin and Lavelle, 1992) as well as indicator of substrate use by microbial communities (Abraham et al., 1998). Most CO_2-enrichment experiments use CO_2 derived from the combustion of fossil fuels, which is depleted in ^{13}C with respect to the background $^{at}CO_2$. Therefore, these are labelling experiments generating plant tissues depleted in ^{13}C concentrations. This approach to C labelling has found many successful applications in the study of C cycling (Ineson et al., 1996; Van Groenigen et al., 2002) and additional application are envisaged (Pataki et al., 2003).

The current experiment was the first attempt to use $\delta^{13}C$ to trace the effects of elevated $^{at}CO_2$ on C flow from litter to isopods and to CO_2 respired by microbial decomposition. We used a broad range of litters derived from three field CO_2-enrichment experiments in the USA. Isopods were chosen for the trial since they are among the main groups of macroarthropods that contribute to plant litter breakdown (Dickinson and Pugh, 1974), and have been previously used to asses the impact of elevated CO_2 on litter palatability (Cotrufo et al., 1998b; Hättenschwiler et al., 1999; David et al., 2001).

2. Material and methods

2.1. Litter samples

Hardwood leaf litters derived from CO_2-enrichment experiments, where the CO_2 used for fumigation was derived from fossil fuel and thus depleted in ^{13}C, were used. The CO_2 fumigation study sites were:

1. Free air carbon dioxide enrichment (FACE) experiment in the Duke Forest (Orange County, North Carolina, USA), with six experimental plots (30 m diameter), three maintained at ambient $^{at}CO_2$ and three constantly enriched with 200 $\mu l\ L^{-1}$ CO_2 above ambient (Hendrey et al., 1999). From this site, litter of *Pinus taeda*; *Liquidambar styraciflua*, *Acer rubrum*, and *Cercis canadensis* was sampled.
2. Oak Ridge FACE experiment at Oak Ridge National Laboratory, TN, USA. At this site, *L. styraciflua* leaf litter was collected from the four experimental plots (25 m diameter), two at ambient $^{at}CO_2$ and two enriched with a CO_2 concentration set point of 535 $\mu l\ L^{-1}$ CO_2 (Norby et al., 2001b).
3. CO_2-enrichment experiment at the Kennedy Space Center, FL, where 16 large (3.5×3.5 m) octagonal open top chambers (OTC) and associated infrastructure for continuous exposure to ambient and elevated $^{at}CO_2$ (350 $\mu l\ L^{-1}$ above ambient) operate in a scrub ecosystem (Drake et al., 1999). Litter from two species: *Quercus myrtifolia* and the N_2 fixer *Galactia elliottii* was collected at this site, from six OTCs (three at ambient $^{at}CO_2$ and three at elevated $^{at}CO_2$).

Between the end of October and the beginning of November 1998, naturally senesced leaf litter was collected from the ground at each experimental site, air-dried and stored in paper bags until used for the palatability trials. Litter was kept separately for each site, species, $^{at}CO_2$ treatment and the experimental plot from which it was collected. At each site ambient and elevated $^{at}CO_2$ experimental plots were paired, so that three pairs of experimental plots were created at the Duke and at the Florida sites and two at the Oak Ridge site.

2.2. Isopod feeding trial

For the feeding trial, isopods of the *Porcellio* sp. were used. On 10 November 1998, over 1000 individuals, ranging in mass from 2 to 30 mg, were collected from a compost heap in a mixed deciduous forest in Edgewater, MD, USA. They were kept in a glass jar, with holes in the lid to aerate the environment, and fed on the same mixed organic material they were living on prior to collection. The day before the incubation started (day 0), they were left on a starvation diet, inside a moistened glass jar, to empty their gut.

For each of the seven litters (i.e. *A. rubrum*, *C. canadensis*, *P. taeda*, *L. styracjflua* from Duke and Oak Ridge, *G. elliottii*, *Q. myrtifolia*), three types of units were established per pair of experimental plots: A, containing 2 g air-dried litter derived from the ambient $^{at}CO_2$ plot; E, with 2 g air-dried litter derived from the enriched $^{at}CO_2$ plot; AE, containing 1 g of air-dried litter derived from the ambient $^{at}CO_2$ plot and 1 g from the paired enriched $^{at}CO_2$ plot.

On day 0 (16 November 1998), 2 g of air-dried intact litter, with petioles removed for broadleaves, was enclosed in a 250 ml plastic beaker (Fischer Scientific, USA), and moistened to 200% water content, by addition of 4 ml deionised H_2O. Units were as described above, with a total of 80 units being established. For *G. elliottii*, only 1 g air-dried leaf litter, and 2 mL H_2O, were used because of insufficient material. A litter sub-sample from each litter type was oven-dried at 70 °C for 72 h, for correction of air-dried mass. All data reported are expressed on a oven-dry weight basis.

On day 1 (17 November 1998), seven isopods were added to each unit (total isopod fresh weight per unit ranged between 23 and 46 mg) and units incubated in a humidified cabinet in the dark at 18 °C. A sample of a few isopods was weighed and frozen at −78 °C, until freeze-dried and used for isotope analyses. On day 2, at a careful check of each unit, a total of 28 isopods were found dead and replaced with live ones. In one unit, three isopods were dead and replaced. In five units, two isopods were replaced, and in the remaining 15 units only one isopod was replaced per unit. A similar check was made on day 3, and 10 more isopods,

all from different units, were found dead and replaced. In all these units isopods died because they were trapped in a film of water. In the few units where excess water (not absorbed by leaves) was observed it was removed from the beakers.

On day 14, isopods were removed from the units, counted, weighed, and freeze-dried. In several units, only five or six isopods were recovered, probably because isopods feed on each other. In seven complete units (i.e. A, E and AE) it was possible to recover faecal pellets: from one set with *P. taeda* litter, one with *A. rubrum*, all three with *C. canadensis* and two with *L. styraciflua*; these pellets were collected and freeze-dried. From all units, litter was oven dried at 40 °C and weighed for determination of mass loss. The dried isopods, litter and faecal pellet samples were stored until used for $\delta^{13}C$ analyses.

2.3. Microbial feeding trial

For the microbial feeding trial, the litters derived from all sites listed above were used, and incubation units established following the same scheme as for the isopods trial, with one A, one E and one AE unit for each pair of experimental plots, for a total of 60 units. A known amount (ranging from 0.05 to 0.2 g, depending on species) of air-dried leaf litter of each litter type was incubated, on the top of a 1 cm layer of glass-wool, in a 7 mL plastic vessel; litter was moistened to 100% water content by addition of deionised water in the vessels. Vessels were closed with a moist cotton cup to minimize water loss from litter and incubated in the dark at 15 °C.

On five occasions during the 30 days of incubation, the $\delta^{13}C$ of the CO_2 respired from litter was measured. On the day of sampling, each vessel had CO_2 removed from headspace by circulating CO_2-free air and then closed airtight. After incubation for 2 h at 15°C, 2 mL air samples were collected from the vessel, by a syringe inserted in the septum of the lid, and injected in a gas-chromatograph coupled with a mass spectrometer (Finnigan MAT 252) for the determination of $\delta^{13}C$-CO_2. Following measurements, deionised water was added to each vessel to replace eventual water loss, determined gravimetrically.

2.4. Chemical analyses

All litter samples were analysed for their C and N concentrations in an elemental analyser (Carlo Erba NA 1500).

The C isotopic composition of leaf litter, isopods and faeces was measured on a mass spectrometer coupled with an elemental analyser (Finnigan Delta plus). For litter, a sub-sample from each type was milled and 2 mg weighed in a tin cup for the analyses of $\delta^{13}C$. Similarly, 2 mg of faecal material from each unit were placed in a tin cup and analysed for $\delta^{13}C$. For isopods, two isopods from each unit were placed in a tin cup and analysed for $\delta^{13}C$.

2.5. Data analyses

The mass balance of stable isotope was applied to the data on faecal pellets and isopods to determine isopods' feeding preferences. In the AE-mixture unit, the fraction of pellets derived from A litter (f_A) was calculated as follow:

$$f_A = (\delta_E - \delta_{AE})/(\delta_E - \delta_A) \quad (1)$$

with δ_{AE}, δ_A and δ_E being, respectively, the $\delta^{13}C$ of faecal pellets from the paired AE, A and E unit. As a consequence the fraction derived from E litter was $f_E = (1 - f_A)$.

A similar approach was applied to calculate the fraction of the isopod's body derived from the litter (f_L) on which they were fed

$$f_L = (\delta_{II} - \delta_{IF})/(\delta_{II} - \delta_L) \quad (2)$$

with δ_{II}, δ_{IF} being the $\delta^{13}C$ of isopods prior to and after incubation and δ_L the $\delta^{13}C$ of litter. In the AE-mixture unit a weighted average of A and E litter $\delta^{13}C$ values was used as δ_L.

Data were processed using SAS system v. 8.1 (SAS inc.) and Origin v. 6. Statistically significant differences among litter species and $^{at}CO_2$ treatment, for mass loss and fraction of the isopod's body derived from litter were assessed applying one and two way ANOVA.

Paired *t*-tests were applied to asses significance differences between $\delta^{13}C$ values of litter and those of faecal pellets and respired CO_2. Values were paired per unit.

3. Results

3.1. Litter quality and $\delta^{13}C$

The elevated $^{at}CO_2$ treatment did not affect the N concentration of the litter examined, either significantly or consistently (Table 1). Litter used in this study exhibited a wide range of N concentrations, with values of 1.44% N in the N_2-fixer *G. elliottii*, to 0.42% N in the coniferous *P. taeda* litter, which consequently resulted in a wide range of litter C/N ratios, from 34 to 125, respectively, for the same two species (Table 1). As expected, litter under enriched $^{at}CO_2$ had a lower $\delta^{13}C$ than the respective litter under ambient $^{at}CO_2$ for all species and study sites. However, variability among experimental plots of the same species and study site was observed for the $\delta^{13}C$ of elevated $^{at}CO_2$ litter, with the highest change of 7‰ being observed for the *P. taeda* litter produced in different rings of the FACE facility at Duke (Table 1).

3.2. Isopods $\delta^{13}C$

The C isotope ratios of the isopods prior to the incubation averaged -22.8 ± 0.06‰. After 14 days incubation their C isotope composition had changed towards that of

Table 1
Carbon isotope ratios ($\delta^{13}C$), and C and N concentrations of hardwood leaf litter grown under ambient and elevated atmospheric CO_2 at the three different experimental sites

Site	Species	$\delta^{13}C$ (‰)		C (%)		N (%)		C/N	
		Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Duke FACE									
	Pinus taeda	−29.3±0.3	−39.0±2.2	49.7±0.3	49.9±0.4	0.42±0.07	0.46±0.02	125±20	109±4
	Cercis canadensis	−31.6±0.3	−44.4±0.4	45.7±0.5	44.0±0.6	0.98±0.06	0.90±0.16	47±3	52±11
	Acer rubrum	−31.1±0.3	−42.7±0.9	45.8±0.3	47.5±0.2	0.54±0.09	0.58±0.10	92±18	88±16
	Liquidambar styraciflua	−30.3±0.5	−43.7±0.6	45.5±0.9	44.8±0.7	0.63±0.03	0.53±0.04	73±4	85±5
ORNL-FACE									
	Liquidambar styraciflua	−29.1±0.1	−42.1±1.8	45.3±0.5	45.3±1.0	0.74±0.11	0.92±0.2	62±11	53±13
SERC-OTC									
	Galactia elliottii	−29.6±0.1	−44.8±0.8	48.2±0.2	48.5±0.2	1.44±0.02	1.38±0.21	34±1	37±6
	Quercus myrtifolia	−30.2±0.3	−44.7±0.6	49.2±0.1	47.3±1.3	0.64±0.08	0.66±0.05	79±9	72±8

Values are means with standard errors; $n=3$ for Duke and SERC and $n=2$ for ORNL.

the litter on which they fed (Table 2), with a mean $\delta^{13}C$ of −24.6, −27.7 and −26.6, respectively, for isopods raised on A, E and AE litter. Also, the faecal pellets produced by the isopods during incubation showed a C isotope composition that did not statistically differ (paired *t*-test) from that of the litter on which the isopods were fed (Fig. 1). Using Eq. (1), the relative composition of faecal pellets in the mixture AE unit was assessed. Because of the limited number of units sampled, a thorough statistical analyses could not be performed. However, in the faecal pellets retrieved from the *A. rubrum*, *P. taeda* and *L. styraciflua* (from Oak Ridge) mixture units, 79, 81 and 85%, respectively, of the C was derived from the A litter, whereas in those retrieved from the *C. canadensis* units only 31±8.5% of their C was derived from the A litter.

3.3. Litter palatability

The fractions of C in the isopods derived from ambient, elevated or mixed AE litter (f_L), calculated accordingly to Eq. (2), are reported in Fig. 2. Independently of species, litter raised under ambient or elevated $^{at}CO_2$ appeared to be equally palatable to isopods, and no significant treatment difference was observed for any species, except for the *Q. myrtifolia* E litter which was eaten more than the respective A litter (Fig. 2). Independently of the litter source, at the end of the experiment a significant percentage of the isopod body was composed of C derived from the litter (Fig. 3a). Isopod C assimilation varied among the different litter species and was significantly correlated with total C loss from the litter during the incubation, although it could explain only a minor fraction of the C loss (Fig. 3b). During the 2 weeks of incubation, litter mass loss values ranged from 2 to 14% depending on the species (data not shown). Statistically significant differences were found among different species, but no effects of elevated $^{at}CO_2$ were observed, and for all the species, E litter decomposed at a similar rate to A litter. No difference between $^{at}CO_2$ treatments was observed for the AE-mixture units.

3.4. $\delta^{13}C$ of CO_2

To evaluate the potential for differential microbial decomposition of litter produced under ambient and elevated $^{at}CO_2$ treatments, we measured the amount of

Table 2
Carbon isotope ratios ($\delta^{13}C$) of adult isopods of the *Porcellio* sp. fed on leaf litter grown under ambient (A) and elevated (E) atmospheric CO_2 or on a mixture of the two litters (AE) for 14 days

Site	Species	$\delta^{13}C$ (‰)		
		A	E	AE
Duke FACE				
	Pinus taeda	−23.9±0.2	−26.6±0.2	−25.4±0.3
	Cercis canadensis	−26.7±0.5	−30.6±0.5	−31.4±1.0
	Acer rubrum	−25.4±0.3	−28.1±0.3	−26.9±1.0
	Liquidambar styraciflua	−25.3±0.1	−30.2±0.1	−27.9±1.0
ORNL-FACE				
	Liquidambar styraciflua	−24.4±0.2	−28.2±0.2	−25.3±0.1
SERC-OTC				
	Galactia elliottii	−23.4±0.3	−24.7±0.3	−24.8±0.4
	Quercus myrtifolia	−22.9±0.1	−25.6±0.1	−24.7±0.4

Values are mean with standard errors ($n=3$). Before the incubation, the $\delta^{13}C$ of the isopods was −22.8±0.06‰.

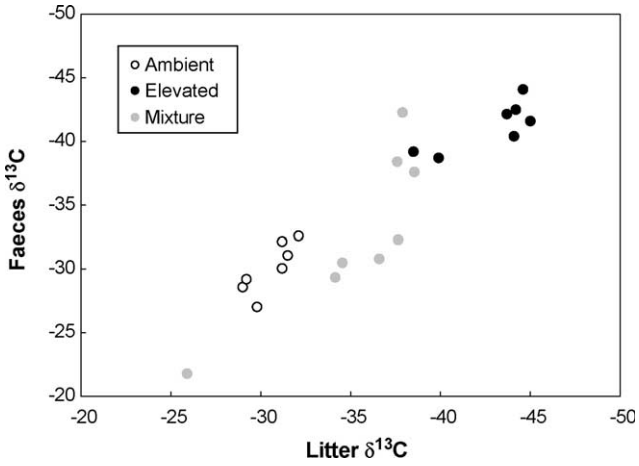

Fig. 1. Values of $\delta^{13}C$ of faecal pellets produced by isopods plotted against $\delta^{13}C$ of the litter on which the isopods were fed ($n=24$; $R=0.93$; $P<0.0001$). No statistical differences between $\delta^{13}C$ of faecal pellets and corresponding litters were measured by a paired t-test.

litter decomposed over a 30-day period and the carbon isotope ratios of the CO_2 released from this respiratory activity.

On average, 16.3% of the litter was decomposed during the 30-day experimental period. Litter produced under either ambient- or elevated-$^{at}CO_2$ conditions decomposed at the same rate in our experiment (Mann–Whitney $U'=15.0$, $P=0.69$). Additionally, there were no significant changes in the mean C isotope ratios of litter between the beginning and end of the experimental period (Mann–Whitney $U'=906.50$, $P=0.83$).

A significant linear relationship between the C isotope ratio of the CO_2 released by microbial decomposition of the litter and the substrate isotopic composition was observed in all units (Fig. 4). There was a tight relationship between the isotopic composition of CO_2 evolved during microbial decomposition and that of the substrate. Yet, the regressed slope of each of the relationships was less than unity, implying the potential for differential fractionation during decomposition (Table 3). However, only the observations on day 30 had a slope that was significantly less than 1 ($P<0.05$).

We then calculated the potential for C isotope discrimination during microbial decomposition among individual treatments (A, E, AE) on different days following litter incubation. The calculated C isotope discrimination was always significantly greater than 0 (Table 4), implying some C isotope fractionation during decomposition. There was a tendency for microbial decomposition of litter produced under elevated CO_2 to fractionate more than for litter produced under ambient conditions and for this fractionation to increase over time. This tendency is consistent with the slope data (Table 3), which yielded slopes apparently less than 1 and with the shallowest slope on day 30. However, because of the limited sample sizes and large standard deviations, none of the calculated C isotope discrimination values were significantly different from each other at the 0.05 level (although the day 30 ambient $^{at}CO_2$ versus elevated $^{at}CO_2$ comparison gave a t value of 2.68, $P=0.055$, df = 19).

The mass balance of stable isotopes was applied to calculate the relative contribution of the CO_2 derived from A and E litters to the CO_2 respired from the AE units. Results were somewhat different among individual units and a clear trend did not appear, suggesting no preference

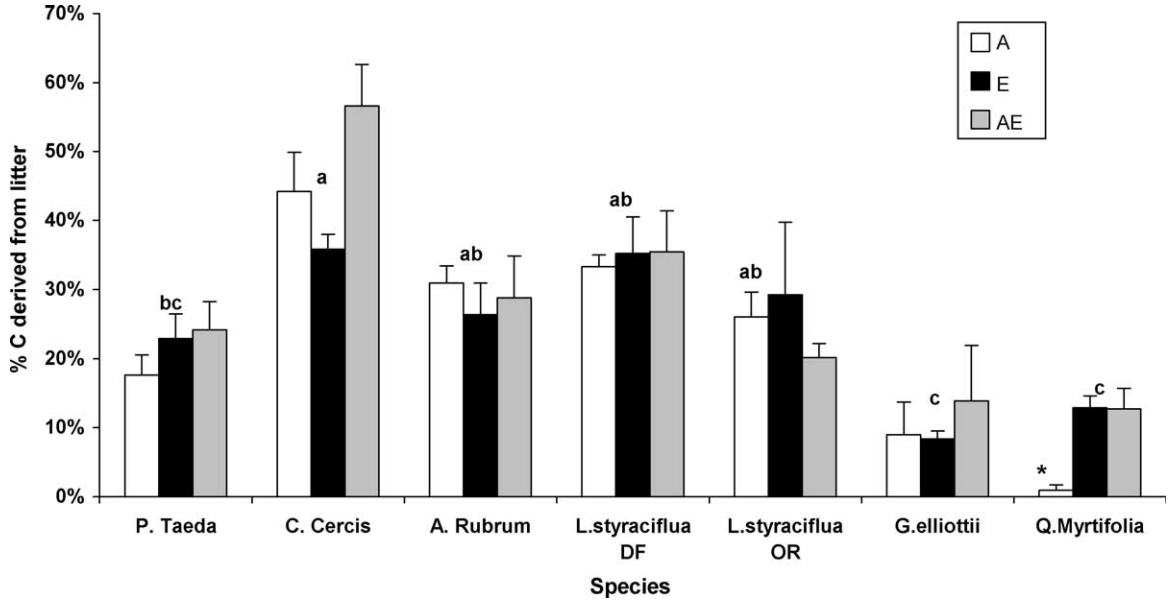

Fig. 2. Fraction of C in the isopods derived from ambient (A), elevated (E) or mixed (AE) litter and expressed as a percentage of total C in the isopods. Values are mean ($n=3$) with SE shown by the error bars. Different letters indicate significant ($P<0.05$) differences among species, after an ANOVA plus a posteriori Tukey's test. Within species, significant difference between treatments is shown by * ($P<0.05$).

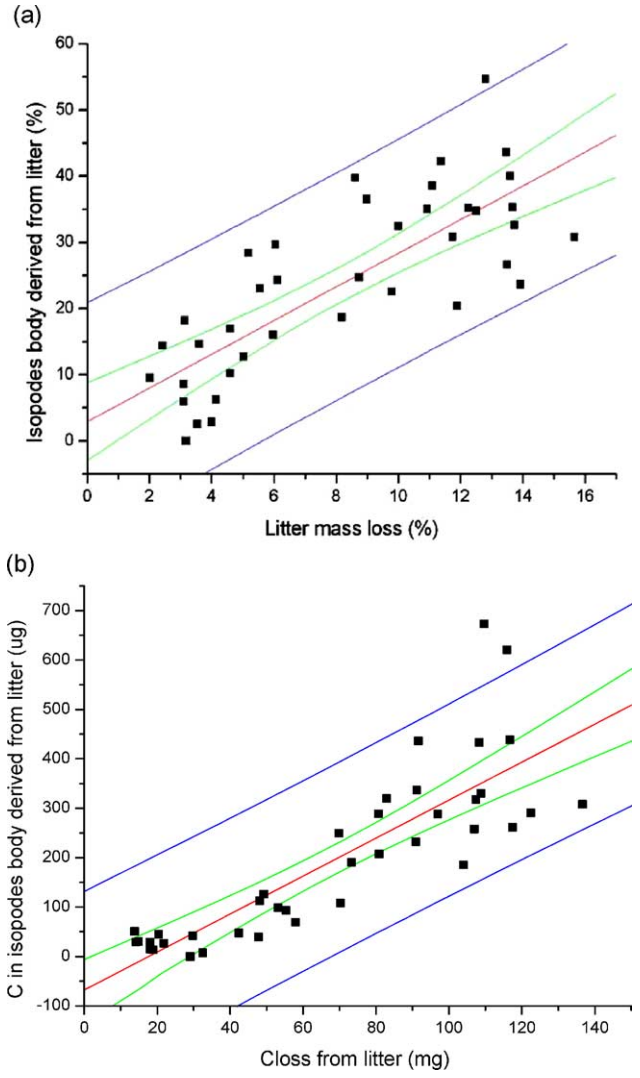

Fig. 3. Contribution of litter C to the isopod's body in relation to litter mass loss, after the 14 days of incubation, expressed as percentages (a) and in terms of C amounts (b). Linear fits with the 95% confidence limit and 95% prediction limit are shown ($n=40$; $P<0.0001$).

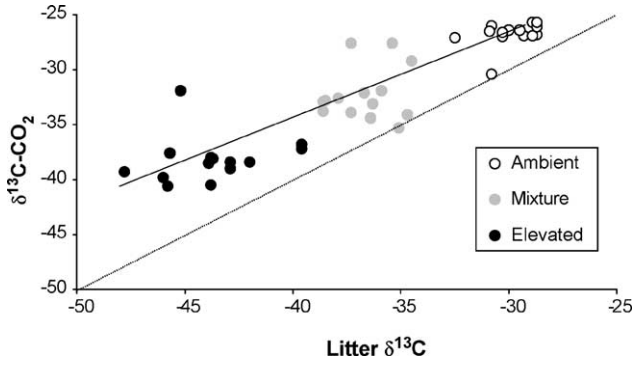

Fig. 4. Values of δ^{13}C-CO_2 respired from litter in relation to the δ^{13}C of the litter measured during the 30 days of incubation. Values refer to individual units and, for the respiration, are means of the five samplings. The regression line is shown ($y=0.77x-3.9$; $R=0.91$; $P<0.0001$), and compared to the 1:1 line. The two series are significantly different (paired t-test $P<0.0001$).

Table 3
A comparison of the slope (±standard deviation), intercepts, R^2 value, and probabilities of significance for the relationships between the C isotope ratio of CO_2 evolved by microbial decomposition and the isotope ratio of litter produced under ambient CO_2, elevated CO_2, and a 50:50 mixture treatment

Days following incubation initiation	Slope	Intercept	P	R^2	P
Day 1	0.88±0.10	0.2	0.001	0.852	0.952
Day 6	0.92±0.09	−0.7	0.001	0.888	0.834
Day 10	0.93±0.09	0.2	0.001	0.887	0.952
Day 30	0.83±0.08	−2.1	0.001	0.882	0.519

Table 4
The calculated C isotope fractionation associated with microbial decomposition of litter over time for treatments involving litters produced under ambient (A) or elevated (E) CO_2, and a 50:50 mixture (AE)

Treatment	Day 6	Day 10	Day 30
A	2.3±1.0	3.2±1.1	3.1±0.9
AE	2.7±2.0	2.8±2.6	4.3±2.6
E	2.3±2.9	3.1±2.4	5.5±2.4

for ambient- or elevated-$^{at}CO_2$ litter by the microbes (data not shown).

4. Discussion

Carbon dioxide enrichment experiments are a useful source of ^{13}C-labelled material, thus opening a broad range of applications for studies on the effects of elevated CO_2 on C cycling (Pataki et al., 2003), which will certainly contribute to the advancement of the understanding of future changes in C cycling in terrestrial ecosystems.

In the current experiment, δ^{13}C values of *P. taeda* needles generated under elevated $^{at}CO_2$ in different experimental plots of the same FACE experiment, showed up to a 7‰ variation between plots. Smaller between-plot differences were measured for the other litter species, although these were greater than the variations in δ^{13}C measured in the corresponding ambient litter (Table 1). Differences were also observed between litter of different species derived from the same experimental plot. In pine litter, this variation may have been caused by the fact that needle litter is derived from needles generated over a range of years. Thus pine litter may have carried the δ^{13}C signal of the atmospheric CO_2 previous to the treatment. However, the variation may also be related to differential distribution of the $^{at}CO_2$ enrichment within the plot and along canopy height. In this respect, plant δ^{13}C is shown to be a useful tool to assess homogeneity in the CO_2 enrichment of experimental plots.

This study confirmed that stable C isotopes enable C flow to be traced along the decomposer food chain, from the litter to the consumers' biomass and their bioproducts (i.e. faeces and CO_2), thereby opening a broad range of applications in

soil ecology. The ^{13}C label was, in fact, clearly transferred from the litter source to the isopods' bodies (Fig. 2) and faeces (Fig. 1), even when litter and isopods' biomass differed only slightly in their original $\delta^{13}C$ values (i.e. when ambient litter was used). We highlight here possible improvements for further applications of this methodology, which we highly encourage. First, since the greater the initial difference in $\delta^{13}C$ between source material and feeder the higher the chance of making an accurate estimation of C flow, we suggest raising the feeders on a litter source different in $\delta^{13}C$, as for example on C4 litter (i.e. with a $\delta^{13}C$ of around $-14‰$), prior to using them in the feeding trial. In addition, since the insect exoskeleton has a lower turnover rate than the inner body parts, it may carry the $\delta^{13}C$ signal of the original diet, diluting the signal from the newly assimilated C. To overcome this problem, it may be advisable to analyse only the inner body parts (i.e. gut) for $\delta^{13}C$. Faeces were shown to be a sensitive material to use when animal feeding is assessed by means of isotopes, and great care should be taken in collecting and analysing them. Finally, isopods were confirmed to be omnivorous (Dickinson and Pugh, 1974), with cannibalistic behaviour, feeding on other isopods. Thus, individual organisms should be used in incubations for feeding trials on plant litter material.

Despite these problems, which limited our ability to quantify preferential feeding in the mixed units, we could accurately determine the amount of C in the isopods' body derived from the litter on which they fed (Fig. 2). We also showed that this was positively correlated to litter mass loss, thus confirming the important role of isopods in promoting litter decomposition (Swift et al., 1979). Independently of the $^{at}CO_2$ treatment, litter palatability to isopods was strongly species specific, as also observed in previous studies (David et al., 2001; Rushton and Hassall, 1983), although quality factors other than the litter N concentration were here responsible for these differences.

Overall, elevated $^{at}CO_2$ did not significantly affect the N concentration and the C-to-N ratios of the hardwood leaf litter used in this study (Table 1). Somewhat surprising was the observation that elevated $^{at}CO_2$ appeared to increase the N concentration in *L. styraciflua* from the FACE experiment in Oak Ridge but not in the Duke study. However, the effects of elevated $^{at}CO_2$ on litter quality can be strongly dependent on the experimental system and on growth conditions (Norby et al., 2001a).

As for litter N, the elevated $^{at}CO_2$ treatment did not affect the palatability to isopods or microbial respiration of any of the litters used (except *Q. myrtifolia* litter derived from the OTCs in the Florida CO_2-enrichment experiment, Fig. 2). The analyses of faecal pellets gave evidence of increased feeding on A litter compared to E litter for *P. taeda*, *A. rubrum* and *L. styraciflua* litter, whereas the opposite was observed for *C. canadensis*. Unfortunately, no faecal pellets were retrieved from the units with *Q. myrtifolia* litter. Previous studies assessing the effects of elevated $^{at}CO_2$ on the palatability of leaf litter from hardwoods (Cotrufo et al., 1998; Hättenshwiler et al., 1999) and grasses (David et al., 2001) brought us to the conclusion that elevated $^{at}CO_2$ may have opposite effects on the consumption and assimilation rate of litter, mostly depending on the litter species (i.e. with high or low initial litter palatability) and on the stage of decomposition (David et al., 2001). The observation that a low-quality litter such as *Q. myrtifolia*, was more acceptable to isopods when grown under elevated $^{at}CO_2$ than under ambient $^{at}CO_2$ appears to confirm the hypothesis that, in litter grown under elevated $^{at}CO_2$, inhibitory factors are lost at very early stages during decomposition, leading to higher consumption rates than for ambient litter, irrespective of the N content of the litter (David et al., 2001).

In our experiment, the $\delta^{13}C$ of the respired CO_2 was significantly higher than that of the litter (Fig. 4), possibly indicating preferential degradation of substrates enriched in ^{13}C as compared to the overall litter. Unfortunately, we did not identify the $\delta^{13}C$ of specific compounds nor their relative concentrations at the beginning and at the end of the incubation. However, it is well documented that litter C-compounds differ in their isotopic composition, with more negative values being associated with lignin and higher values with more decomposable fractions such as non-acid detergent fibre and cellulose (Fernandez et al., 2003; Schweizer et al., 1999).

With this study, we further demonstrated that the use of isotopically labelled litter can strongly contribute to the advancement of the understanding of litter decomposition and of the quantification of C fluxes in the process.

Acknowledgements

This work was supported by a Smithsonian Visiting Research Scholarship. We are grateful to Rich Norby and William Schlesinger for access to FACE facilities and to Adrien Finzi and Roser Matamala for their help during litter collection.

References

Abraham, W.R., Hesse, C., Oliver, P., 1998. Ratios of carbon isotopes in microbial lipids as indicator of substrate usage. Applied and Environmental Microbiology 84, 4202–4209.

Cotrufo, M.F., Ineson, P., Scott, A., 1998a. Elevated CO_2 reduces the nitrogen concentration of plant tissues. Global Change Biology 4, 43–54.

Cotrufo, M.F., Briones, M.J.I., Ineson, P., 1998b. Elevated CO_2 affects field decomposition rate and palatability of tree leaf litter: importance of changes in substrate quality. Soil Biology & Biochemistry 30, 1565–1571.

David, J.F., Malet, N., Couteaux, M.M., Roy, J., 2001. Feeding rates of the woodlouse *Armadillium vulgare* on herb litters produced at two levels of atmospheric CO_2. Oecologia 127, 343–349.

Dickinson, C.H., Pugh, G.J.F., 1974. Biology of plant litter decomposition. Academic Press, London.

Drake, B.G., Berry, J., Bunce, J., Dijkstra, P., Farrar, J., Gifford, R., Koch, G., Lambers, H., Gonzalez-Mehler, M., Azcon-Bieto, K., Siedow, J., Wullschleger, S., 1999. Does elevated atmospheric CO_2 concentration inhibit mitochondrial respiration in green plants?. Plant, Cell and Environment 22, 649–657.

Fernandez, I., Mahieu, N., Cadisch, G., 2003. Carbon isotope fractionation during decomposition of plant materials of different quality. Global Biogeochemical Cycles 17 (3), 1–11.

Hättenshwiler, S., Bühler, S., Körner, C., 1999. Quality, decomposition and isopod consumption of tree litter produced under elevated CO_2. Oikos 85, 271–281.

Hendrey, G.H., Ellsworth, D.S., Lewin, K.F., Nagy, J., 1999. A free-air enrichment system for exposing tall forest vegetation to elevated atmospheric CO_2. Global Change Biology 5, 293–309.

Ineson, P., Cotrufo, M.F., Bol, R., Harkness, D.D., Blum, H., 1996. Quantification of soil C input under elevated CO_2: C_3 plants in a C_4 soil. Plant and Soil 187, 345–350.

Martin, A., Lavelle, P., 1992. Effect of soil organic matter quality on its assimilation by *Millsonia animala*, a tropical geophagous earthworm. Soil Biology & Biochemistry 12, 1535–1538.

Norby, R.J., Cotrufo, M.F., Ineson, P., O'Neill, E.G., Canadell, J.G., 2001a. Elevated CO_2, litter quality and decomposition: a synthesis. Oecologia 127, 153–165.

Norby, R.J., Todd, D.E., Fults, J., Johnson, D.W., 2001b. Allometric determination of tree growth in a CO_2-enriched sweetgum stand. New Phytologist 150, 477–487.

Pataki, D.E., Ellsworth, D.S., King, J.S., Leavitt, S.W., Lin, G., Pendall, E., Siegwolf, R., van Kessel, C., Ehleringer, J.R., 2003. Tracing changes in ecosystem function under elevated CO_2. Bioscience 53, 805–818.

Peñuelas, J., Estiarte, M., Llusia, J., 1997. Carbon-based secondary compounds at elevated CO_2. Photosynthetica 33, 313–316.

Radajewski, S., Ineson, P., Parekh, N.R., Murrell, J.C., 2000. Stable-isotope probing as a tool in microbial ecology. Nature 403, 646–649.

Roth, S.K., Lindroth, R.L., 1995. Elevated atmospheric CO_2: effects on phytochemistry, insect performance and insect–parasitoid interactions. Global Change Biology 1, 173–182.

Rushton, S.P., Hassall, M., 1983. Food and feeding rates of the terrestrial isopod *Armadillidium vulgare* (Latreille). Oecologia 57, 257–261.

Schweizer, M., Fear, J., Cadish, G., 1999. Isotopic (^{13}C) fractionation during plant residue decomposition and its implications for soil organic matter studies. Rapid Communication in Mass Spectrometry 13, 1284–1290.

Swift, M.J., Heal, O.W., Anderson, J.M., 1979. Decomposition in Terrestrial Ecosystems. Blackwell, Oxford.

Van Gronigen, K.J., Harris, D., Horwath, W.R., Hartwig, U.A., van Kessel, C., 2002. Linking sequestration of ^{13}C and ^{15}N in aggregates in a pasture soil following 8 years of elevated atmospheric CO_2. Global Change Biology 8, 1094–1108.

Vu, J.C.V., Allen, L.H., Bowes, J.R.G., 1989. Leaf ultrastructure, carbohydrates and protein of soybeans grown under CO_2 enrichment. Environmental & Experimental Botany 29, 141–147.