Short communication

Natural abundance of ^{13}C in leaf litter as related to feeding activity of soil invertebrates and microbial mineralisationHana Šantrůčková^{a,b,*}, M.I. Bird^c, J. Frouz^b, V. Šustr^b, K. Tajovský^b^aFaculty of Biological Sciences, University of South Bohemia, Na sádkách 7, CZ-370 05, České Budějovice, Czech Republic^bInstitute of Soil Biology AS CR, Na sádkách 7, CZ-370 05, České Budějovice, Czech Republic^cResearch School of Earth Sciences, Australian National University, Canberra, ACT 0200, Australia

Accepted 16 February 2000

Feeding activity of soil invertebrates and microbial respiration are shown to change natural abundance of ^{13}C of organic matter and respired CO_2 in a range of 2%.

The ^{13}C in soil organic matter (SOM) and that of CO_2 released from soil have been used to study SOM turnover, but knowledge of the factors affecting the natural abundance of ^{13}C in SOM and soil CO_2 remains limited (Buchmann et al., 1998). The stable-isotopic composition ($\delta^{13}\text{C}$ value) of SOM and CO_2 respired from soil should reflect that of the local plant cover (Deines, 1980). However, the $\delta^{13}\text{C}$ value of SOM exhibits systematic differences from the $\delta^{13}\text{C}$ value of C derived from local vegetation, ranging from -6.1 to $+4.4\%$ (e.g. Nadelhoffer and Fry, 1988; Melillo et al., 1989; von Fischer and Tieszen, 1995). The difference between the $\delta^{13}\text{C}$ of SOM and that of soil CO_2 has been found to vary from -3.2 to $+2.1\%$ (e.g. Readon et al., 1979; Dörr and Münnich, 1980; Parada et al., 1983; Hesterberg and Siegenthaler, 1991).

One source of the observed shift in the $\delta^{13}\text{C}$ value of SOM and CO_2 could be isotope effects which occur during the decomposition of plant litter by soil microorganisms and invertebrates. A shift in the isotopic composition of SOM in a range from -3.7 to $+1.4\%$ due to aerobic microbial degradation of SOM has been observed (Šantrůčková et al., 2000). Isotopic effects which may result from the activities of soil in-

vertebrates have not been widely studied and the only available data are concerned with earthworms (Martin et al., 1992a,b; Schmidt et al., 1997). Isotopic effects by soil heterotrophs could be induced by (i) discrimination during metabolism and (ii) by the selective consumption and utilisation of chemical compounds having $\delta^{13}\text{C}$ values deviating from that of the plant litter.

- (i) Metabolism of organisms might favour ^{13}C -enrichment of SOM because catabolic reactions prefer molecules which are ^{13}C -depleted, while those which are ^{13}C -enriched tend to be utilised in the production of biomass and the end-products of metabolism (DeNiro and Epstein, 1978; Blair et al., 1985; Schmidt and Gleixner, 1998). This should lead to a ^{13}C -depletion of respired CO_2 and to a ^{13}C -enrichment in organic material remaining in the soil.
- (ii) Soil heterotrophs selectively use organic materials which differ in age, origin, degree of decomposition and, therefore, in chemical and isotopic composition (Gunnarson and Tunlid, 1986; Griffiths et al., 1989; Hopkins et al., 1998). If easily available compounds, which are high in ^{13}C , are utilised, the remaining organic material will be ^{13}C -depleted. Conversely, if more complex low- ^{13}C substances (e.g. lipids or lignin) are utilised, remaining organic material will become ^{13}C -enriched (Deines, 1980; Ågren et al., 1996).

* Corresponding author. Tel.: +420-38-777-5764; fax: +420-38-53-00-248.

E-mail address: hana@upb.cas.cz (H. Šantrůčková).

Our objectives were to determine (i) the relationship between the isotopic composition of leaf litter (diet)

and the excrement of soil invertebrates and (ii) to compare isotopic effects of the microbial decomposition of leaf litter and excrement.

In a laboratory experiment we analysed the food and excrement of litter feeding invertebrates with reference to the $\delta^{13}\text{C}$ value of total carbon and respired CO_2 . To obtain excrement samples, litter was fed (as described by Frouz and Šustr, 1996) to larvae of two bibionid species (*Bibio marci*, Linné, 1758 and *Bibio pomonae*, Fabricius, 1775) and to one species of terrestrial isopod (*Armadillo officinalis*, Duméril, 1816). *B. marci* was fed on hornbeam (*Carpinus betulus*, L.) and oak (*Quercus robur*, L.) leaves, *B. pomonae* was fed on poplar (*Populus nigra*, L.) leaves and *A. officinalis* was fed on litter from deciduous forest containing mainly hornbeam and oak (litter I) and the litter from deciduous forest mixed with apple flakes (litter II). Excrement was collected every second day for a period of 14 days, pooled and subsampled. The food and excrement (Table 1) were analysed for $\delta^{13}\text{C}$ and used for the measurement of microbial respiration. About 1 g of food or excrement was mixed with 10 g of sterile, C free silica sand (<0.5 mm) and with 3 ml of sterile water, and incubated for 3 days in a closed system at 20°C; three replicates were used. CO_2 was trapped into bicarbonate-free NaOH. At the end of the experiment, the trapped CO_2 was released by acid addition and purified cryogenically. Food and excrement were dry combusted at 900°C with CuO and silver wire in sealed Quartz tubes (Boutton et al., 1983) and the CO_2 produced was purified cryogenically. The amount of purified CO_2 released by acid addition and dry combustion was determined manometrically in a calibrated volume and the isotopic ratios for $\delta^{13}\text{C}$ were measured using a Finnigan MAT-251 mass spectrometer. All the measurements were performed in duplicate, and the results are reported as parts per thousand (‰) deviations from the defined international V-PDB standard.

The precision of the analyses was 0.1‰ and the standard deviation of the duplicates did not exceed 0.5‰.

The significance of differences between respiration of litter and excrement was evaluated using confidence limits ($n = 3$, $P = 0.05\%$). Carbon isotopic discrimination caused by animal feeding and microbial mineralisation is described using a fractionation factor Δ (Farquhar and Richards, 1984):

$$\Delta_{R/P} = (\alpha_{R/P} - 1) = (\delta_R - \delta_P)/(1 + \delta_P)$$

where α is

$$\alpha_{R/P} = R_R/R_P,$$

R_R is the $^{13}\text{C}/^{12}\text{C}$ molar ratio of the reactant (R) and R_P is that of the product (P). If the fractionation factor, Δ , is positive then the product is depleted in ^{13}C relative to the reactant. Negative value of Δ indicates an enrichment of the product compared to the reactant. The effect of animal feeding is described by $\Delta_{\text{food/excr}}$ and the effect of microbial mineralisation by $\Delta_{\text{substr}/\text{CO}_2}$ (subscripts denote leaf litter and excrement, respectively).

Excrement was ^{13}C -depleted relative to food (Fig. 1). The fractionation factor, $\Delta_{\text{food/excr}}$, ranged from +0.6 to +0.1‰ (Table 2). It is impossible to uniquely distinguish between the effects of animal metabolism and that of selective consumption and utilisation of organic compounds on the basis of these results. However, the excrement would be ^{13}C -enriched if affected only by the metabolism of invertebrates. ^{13}C -depletion of excrements indicates that an effect of selective utilisation of organic compounds rich in ^{13}C (protein, pectin, carbohydrates) was larger than that which would be induced by metabolism. The results are consistent with the rapid and preferential loss of polysaccharides during passage of food through the gut of dipteran larva and other insects (Hopkins et al., 1998). DeNiro

Table 1

Content of ash (%) and C_{org} (%), expressed on an ash-free dry mass basis) and C-to-N ratio of leaf litter (food) and excrement, and the amount of CO_2 ($\mu\text{g C gC}^{-1} \text{ h}^{-1}$) respired during mineralisation of leaf litter and excrement

Animal species	Food excrement	Ash (%)	C_{org} (%)	C-to-N ratio	CO_2 ($\mu\text{g C gC}^{-1} \text{ h}^{-1}$)
<i>Bibio marci</i>	Hornbeam leaves	34.4	45.6	25.0	132.2 ± 8.1
	Excrements	37.8	41.9	22.9	154.5 ± 2.1
<i>Bibio marci</i>	Oak leaves	29.7	51.6	18.6	229.2 ± 12.5^a
	Excrements	40.7	51.1	20.4	104.7 ± 2.5
<i>Bibio pomonae</i>	Poplar leaves	22.3	51.9	13.5	366.0 ± 18.1^a
	Excrements	38.0	53.4	15.5	42.4 ± 2.9
<i>Armadillo officinalis</i>	Litter I	16.9	46.5	24.8	184.6 ± 6.7^a
	Excrements	41.2	42.3	15.8	36.2 ± 0.9
<i>Armadillo officinalis</i>	Litter II	18.7	49.3	25.5	85.4 ± 3.5^a
	Excrements	23.4	43.0	22.5	66.1 ± 2.3

^a Significant differences between CO_2 evolution from leaf litter and the excrement derived from the litter (confidence intervals, $P = 0.05\%$, $n = 3$).

Fig. 1. $\delta^{13}\text{C}$ of food (leaf litter) and the excrement of soil invertebrates, and the $\delta^{13}\text{C}$ of CO_2 respired during mineralisation of both the food and excrement. Mean values and standard errors are shown.

and Epstein (1978) found that animal body tissue and faeces are ^{13}C -enriched relative to the diet in most cases and that this ^{13}C -enrichment was balanced by a depletion of ^{13}C in the respired CO_2 . The ^{13}C -enrichment of the body tissue was found to be higher than that of the faeces. Taking into account these findings we can speculate that, in our experiment, body tissue of the invertebrates was ^{13}C -enriched relative to their excrement which would mean that their body tissues might be slightly enriched compared to their food. In agreement with isotopic studies of animals (Fry and Sherr, 1988) we found a small between-species variations in $\delta^{13}\text{C}$ within a range of 2‰.

Food origin and composition might influence isotopic effects associated with animal feeding. Fry and Sherr (1988) compiled data from field and laboratory studies where the diet C source was well known. They report that animal tissue $\delta^{13}\text{C}$ values are within $\pm 2\text{‰}$ of their food source. Hentschel (1998) observed a size-dependent variation in the $\delta^{13}\text{C}$ value of deposit-feeding polychaetes due to ontogenetic changes in diet.

Table 2

Fractionation factors for animal feeding ($\Delta_{\text{food/excr}}$) and microbial respiration ($\Delta_{\text{substr}/\text{CO}_2}$). See text for detailed explanation

Animal species	Food excrement	$\Delta_{\text{food/excr}}$	$\Delta_{\text{substr}/\text{CO}_2}$
<i>Bibio marci</i>	Hornbeam leaves		-1.1
	Excrements	+0.3	-0.9
<i>Bibio marci</i>	Oak leaves		-1.6
	Excrements	+0.3	-1.2
<i>Bibio pomonae</i>	Poplar leaves		-1.1
	Excrements	+0.1	-1.7
<i>Armadillo officinalis</i>	Litter I		+0.5
	Excrements	+0.6	-0.6
<i>Armadillo officinalis</i>	Litter II		-0.7
	Excrements	+0.5	-0.5

Animals feeding on C_4 plants were found to be mostly ^{13}C -depleted relative to their food while those feeding on C_3 plants were ^{13}C -enriched (Fry et al., 1978; Haines and Montague, 1979). Martin et al. (1992b) observed that earthworm bodies are enriched relative to soil or plant material by a fractionation factor of -4.4‰ for C_3 soil and -1.02‰ for C_4 soil. The unusually high ^{13}C -enrichment can be explained by the combined effect of animal metabolism and the preferential utilisation of ^{13}C -rich polysaccharides. Isotope fractionation effects have also been observed by Martin et al. (1992a) and Schmidt et al. (1997), but the isotopic composition of the food source was not clearly defined in their experiments. We did not find a large inter-species difference in $\Delta_{\text{food/excr}}$, which is not surprising because of the similarity in diet used for all animals.

CO_2 respired during microbial metabolism is usually depleted relative to the $\delta^{13}\text{C}$ value of the substrate (Blair et al., 1985; Mary et al., 1992). Thus, the $\delta^{13}\text{C}$ value of respired CO_2 observed in our experiment should be lower than the $\delta^{13}\text{C}$ of the C that was consumed by the microorganisms. We found that respired CO_2 is shifted relative to the total organic C by a fractionation factor, $\Delta_{\text{substr}/\text{CO}_2}$, ranging from $+0.5$ to -1.7‰ (Table 2). CO_2 was enriched compared to both litter and excrement in eight out of ten cases (Fig. 1). This indicates that only a fraction of the total organic C was used in mineralisation processes and that the isotope effect associated with the selective use of organic compounds is more pronounced than the effect of metabolism itself. The organic material that was utilised by microorganisms during aerobic incubation, was ^{13}C -enriched relative to the total organic C. This is consistent with the findings that substances that are resistant to microbial degradation are low in ^{13}C while easily decomposable compounds are high in ^{13}C . This leads to a relatively more rapid loss of ^{13}C over ^{12}C during decomposition and, therefore, to a depletion of remaining material in ^{13}C (Benner et al., 1987; Ågren et al., 1996). Our data are not consistent with findings of Wedin et al. (1995) who found an increase in the $\delta^{13}\text{C}$ value of C_3 -plant material during decomposition. This inconsistency might be explained by Wedin et al. (1995) who studied long-term changes while we investigated short-term variations. During long-term experiments the mixing of C remaining after earlier metabolic processing with new microbial products, which are enriched in ^{13}C , becomes more important (Wedin et al., 1995).

We did not find any consistent change in the fractionation factor, $\Delta_{\text{substr}/\text{CO}_2}$, between the food and the excrement, except for *A. officinalis* fed on litter I (Table 2). This indicates that ^{13}C -rich compounds were mineralised in the food and excrement during the incubation, although the amount of available C is lower in

the excrement than in the initial leaf litter (Frouz et al., 1999). The loss of organic material during passage through the gut is evident from lower respiration rate and higher ash content in the excrement relative to the initial food (Table 1). CO₂ released during microbial decay of excrement of *B. pomonae* fed on poplar was more ¹³C-enriched than that of poplar litter. One could speculate that the ¹³C-enrichment is connected with microbial decay of proteinaceous compounds which became available after passage of poplar litter with high content of N compounds (Table 1). Proteins and amino-acids are more enriched than carbohydrates (Deines, 1980).

Our observations suggest that the effect of selective utilisation of ¹³C-enriched compounds by soil heterotrophs is more significant than metabolic effects, and can induce a ¹³C-depletion in processed leaf litter from C₃ plants.

Acknowledgements

The authors acknowledge the Australian Research Council for a Queen Elizabeth II Fellowship to M.I. Bird; Joan Cowley and Joe Cali for assistance with sample preparation and mass spectrometry measurements, Milan Straškraba for valuable comments on the manuscript and David Wardle for English revisions.

References

- Ågren, G.J., Bosatta, E., Balesdent, J., 1996. Isotope discrimination during decomposition of organic carbon: a theoretical analysis. *Soil Science Society of America Journal* 60, 1121–1126.
- Benner, R., Fogel, M.L., Sprague, E.K., Hodson, R.E., 1987. Depletion of ¹³C in lignin and its implications for stable isotope studies. *Nature* 329, 708–710.
- Blair, N., Leu, A., Muñoz, E., Olsen, J., Kwong, E., des Marais, D., 1985. Carbon isotopic fractionation in heterotrophic microbial metabolism. *Applied and Environmental Microbiology* 50, 996–1001.
- Boutton, T.W., Wong, W.W., Hachey, D.L., Lee, L.S., Cabrera, M.P., Klein, P.G., 1983. Comparison of quartz and Pyrex tubes for combustion of organic samples for stable isotope analysis. *Analytical Chemistry* 55, 1832–1833.
- Buchmann, N., Brooks, J.R., Flanagan, L.B., Ehleringer, J.R., 1998. Carbon isotope discrimination of terrestrial ecosystems. In: Griffiths, H. (Ed.), *Stable Isotopes and the Integration of Biological, Ecological and Geochemical Processes*. BIOS Scientific Publishers, Oxford, pp. 203–221.
- Deines, P., 1980. The isotopic composition of reduced organic carbon. In: Fritz, P., Fontes, J.C. (Eds.), *Handbook of Environmental Isotope Geochemistry*, vol. 1. Elsevier, Amsterdam, pp. 329–406.
- DeNiro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42, 495–506.
- Dörr, H., Münnich, K.O., 1980. Carbon-14 and carbon-13 in soil CO₂. *Radiocarbon* 22, 909–918.
- Farquhar, G.D., Richards, R.A., 1984. Isotopic composition of plant carbon correlates with water use efficiency of wheat genotypes. *Australian Journal of Plant Physiology* 11, 539–552.
- von Fischer, J.C., Tieszen, L.L., 1995. Carbon isotope characterisation of vegetation and SOC in subtropical forests in Luquillo, Puerto Rico. *Biotropica* 27, 138–148.
- Frouz, J., Šustr, V., 1996. The impact of drying and rewetting of leaf litter on feeding activity of *Bibio pomonae* (Diptera, Bibionidae) larvae. *Studia Dipterologica* 3, 101–106.
- Frouz, J., Šantrůčková, H., Elhottová, D., 1999. The effect of bibionid larvae feeding on the microbial community of litter and reconsumed excrements. *Pedobiologia* 43, 221–230.
- Fry, B., Joern, A., Parker, P.L., 1978. Grasshopper food web analysis: use of carbon isotope ratios to examine feeding relationships among terrestrial herbivores. *Ecology* 59, 498–506.
- Fry, B., Sherr, E.B., 1988. δ ¹³C measurements as indicators of carbon flow in marine and freshwater ecosystems. In: Rundel, P.W., Ehleringer, J.R., Nagy, K.H. (Eds.), *Stable Isotopes in Ecological Research*. Springer-Verlag, New York, pp. 196–229.
- Griffiths, B.S., Wood, S., Cheshire, M.V., 1989. Mineralisation of ¹⁴C-labelled plant material by *Porcelio scaber* (Crustacea, Isopoda). *Pedobiologia* 33, 355–360.
- Gunnarson, T., Tunlid, A., 1986. Recycling of fecal pellets in isopods: microorganisms and nitrogen compound as potential food source for *Oniscus asellus* L. *Soil Biology & Biochemistry* 18, 595–600.
- Haines, E.B., Montague, C.L., 1979. Food source of estuarine invertebrates analysed using ¹³C/¹²C ratios. *Ecology* 60, 48–56.
- Hentschel, B.T., 1998. Intraspecific variations in δ ¹³C indicate ontogenetic diet changes in deposit-feeding polychaetes. *Ecology* 79, 1357–1370.
- Hesterberg, R., Siegenthaler, U., 1991. Production and stable isotopic composition of CO₂ in a soil near Bern, Switzerland. *Tellus* 43B, 197–205.
- Hopkins, D.W., Chudek, J.A., Bignell, D.E., Frouz, J., Webster, E.A., Lawson, T., 1998. Application of ¹³C NMR to investigate the transformations and biodegradations of organic material by some soil and litter-dwelling insects. *Biodegradation* 9, 423–431.
- Martin, A., Balesdent, J., Mariotti, A., 1992a. Earthworm diet related to soil organic matter dynamics through ¹³C measurements. *Oecologia* 91, 23–29.
- Martin, A., Mariotti, A., Balesdent, J., Lavelle, P., 1992b. Soil organic matter assimilation by a geophagous tropical earthworm based on ¹³C measurements. *Ecology* 73, 118–128.
- Mary, B., Mariotti, A., Morel, J.L., 1992. Use of ¹³C variations at natural abundance for studying the biodegradation of root mucilage, roots and glucose in soil. *Soil Biology & Biochemistry* 24, 1065–1072.
- Mellilo, J.M., Aber, J.D., Linkins, A.E., Ricca, A., Fry, B., Nadelhoffer, K.J., 1989. Carbon and nitrogen dynamics along the decay continuum: plant litter to soil organic carbon. *Plant and Soil* 115, 189–198.
- Nadelhoffer, K.J., Fry, B., 1988. Controls on natural nitrogen-15 and carbon-13 abundances in forest SOC. *Soil Science Society of America Journal* 52, 1633–1640.
- Parada, C.B., Long, A., Davis, S.N., 1983. Stable-isotopic composition of soil carbon dioxide in the Tuscon Basin, Arizona, U.S.A. *Isotope Geoscience* 1, 219–236.
- Readon, E.J., Allison, G.B., Fritz, P., 1979. Seasonal chemical and isotopic variations of soil CO₂ at Trout Creeg, Ontario. *Journal of Hydrology* 43, 355–371.
- Šantrůčková, H., Bird, M.I., Lloyd, J., 2000. Microbial processes and carbon-isotope fractionation in tropical and temperate grassland soils. *Functional Ecology* 14, 108–114.
- Schmidt, O., Scrimgeour, C.M., Handley, L.L., 1997. Natural abun-

- dance of ^{15}N and ^{13}C in earthworms from a wheat and a wheat-clover field. *Soil Biology & Biochemistry* 29, 1301–1308.
- Schmidt, H.-L.H., Gleixner, G., 1998. Carbon isotope effects on key reactions in plant metabolism and ^{13}C -patterns in natural compounds. In: Griffiths, H. (Ed.), *Stable Isotopes and the Integration of Biological, Ecological and Geochemical Processes*. BIOS Scientific Publishers, Oxford, pp. 13–26.
- Wedin, D.A., Tieszen, L.L., Dewey, B., Pastor, J., 1995. Carbon isotope dynamics during grass decomposition and soil organic matter formation. *Ecology* 76, 1383–1392.