Soil Biol. Biochem. Vol. 19, No. 1, pp. 25–30, 1987
Printed in Great Britain

0038-0717/87 \$3.00 + 0.00
Pergamon Journals Ltd

NATURAL ^{13}C ABUNDANCE AS A TRACER FOR STUDIES OF SOIL ORGANIC MATTER DYNAMICS

JÉRÔME BALESDENT and ANDRÉ MARIOTTI
Laboratoire de Biogéochimie des Isotopes Stables, INRA—Université P. et M. Curie, Département de Géologie Dynamique, T.26 4ème étage, 4 place Jussieu, 75252 Paris Cedex 05, France

and

BERNARD GUILLET
CNRS, Centre de Pédologie Biologique, 17 rue N.D. des Pauvres, B.P. 5, 54501 Vandoeuvre les Nancy Cedex, France

(*Accepted* 4 *July* 1986)

Summary—A method for measuring the long- and medium-term turnover of soil organic matter is described. Its principle is based on the variations of ^{13}C natural isotope abundance induced by the repeated cultivations of a plant with a high ^{13}C/^{12}C ratio (C4 photosynthetic pathway) on a soil which has never carried any such plant. The ^{13}C/^{12}C ratio in soil organic matter being about equal to the ^{13}C/^{12}C ratio of plant materials from which it is derived, changing the ^{13}C content of the organic inputs to the soil (by altering vegetation from C3 type into C4 type) is equivalent to a true labelling *in situ* of the organic matter.

Two cases of continuous corn cultivation (*Zea mays*: $\delta^{13}C = -12‰$) on soils whose initial organic matter average δ^{13}C is −26‰ were studied. The quantity of organic carbon originating from corn (that is the quantity which had turned-over since the beginning of continuous cultivation) was estimated using the ^{13}C natural abundance data. After 13 yr, 22% of total organic carbon had turned-over, in the system studied. Particle size fractions coarser than 50 μm on the one hand, and finer than 2 μm on the other, contained the youngest organic matters. The turnover rate of silt-sized fractions was slower.

INTRODUCTION

Most studies on soil organic carbon dynamics depend upon comparisons between plots receiving different levels of organic inputs, radiocarbon dating (Balesdent and Guillet, 1982; O'Brien, 1984), or incorporation of ^{14}C labelled plant material (Jenkinson, 1977). Although it has not been exploited, the stable carbon isotope ^{13}C at natural abundance concentrations can, in some cases, be a good natural tracer of organic inputs to the soil.

The relation between the δ^{13}C ($\delta^{13}C\ ‰ = [(R_{sample}/R_{standard}) - 1]\cdot 1000$, where R is the isotope ratio $^{13}/^{12}$C) of higher plants and their metabolic pathways has been shown by Bender (1971) and Smith and Epstein (1971). The isotope effect induced by the enzymatic reaction in the primary carboxylation is much higher for C3 plants (Calvin cycle) than for C4 plants (Hatch–Slack cycle). So, from atmospheric CO_2 whose isotopic composition is near −7‰, C3 plants present δ^{13}C values ranging from −23 to −40‰, with a most frequent value of about −27‰, whereas C4 plants present δ^{13}C values from −9 to −19‰, with a most frequent value of −12‰ (Smith and Epstein, 1971; Deleens *et al.*, 1974). When comparing plants which exhibit these two major photosynthetic patterns, and grown in the same environment, there is an average difference of 12–14‰ (Bender, 1971). Almost all temperate species and all trees belong to the C3 group. Most of the tropical Chenopodiaceae and Gramineae e.g. *Zea*, *Sorghum*, *Saccharum*, belong to the C4 group. Mineralization and processes associated with humus formation in soils induce only slight variations in ^{13}C abundance. That is the reason why δ^{13}C values of organic matter of soils in cold and temperate climates and of forest soils range from −24 to −29‰, whereas δ^{13}C values of soils under tropical grasslands and savannas range from −12 to −14‰ (Modenesi *et al.*, 1982). Nissenbaum and Kaplan (1972) related δ^{13}C values of −18.2 and −14.8‰ respectively for fulvic and humic acids in an Hawaïan canefield (C4).

Growing C4 plants on a soil which has previously been under C3 vegetation can thus be literally considered as an *in situ* labelling of the organic matter incorporated into the soil. Because of the relative weakness of the signal of this tracer, it can be best used in the case of several successive cultivations. After a time t of cultivation, if A is the ratio $^{13}C/[^{12}C + {}^{13}C]$ of soil carbon at time t, A_0 this ratio for the initial soil at $t = 0$, or for a similar soil under C3 vegetation, A_1 the ratio for the C4 plant, then the percentage x of carbon coming from the C4 plant can be deduced from:

$$A = x/100 \cdot A_1 + (1 - x/100)\cdot A_0 \qquad (1)$$

and, as δ^{13}C is almost exactly a linear function of the ratio $^{13}C/[^{12}C + {}^{13}C]$,

$$\delta \sim (\delta_1 - \delta_0)\cdot x/100 + \delta_0 \qquad (2)$$

Cerri *et al.* (1985) first used this method in order to measure the turnover rate of organic matter in a 50-yr-old canefield, after forest clearing.

If the total carbon content C is known, the absolute quantity X of carbon from C4 plants can be determined ($X = C \cdot x$). The absolute quantity Y of residual carbon from initial soil is $Y = C(1 - x)$. During cultivation, X will increase with time as a function $X(t)$. If the inputs are constant throughout the experiment, dX/dt is the curve of decay of a single input from the C4 plants and the value of this single input (per unit time) will be dX/dt (0). On the other hand, Y will decrease with time as a function $Y(t)$ and will describe the decay of the whole pool of initial carbon from the C3 vegetation. Because this technique involves changing the plant cover of a soil from one species to another, it will most likely change certain conditions of the soil organic regime, such as the input rate of organic carbon to the soil. This may then affect certain parameters such as total soil organic carbon, which would change in time to a new equilibrium value.

MATERIALS AND METHODS

The soil samples studied were obtained from two experimental fields of the Institut National de la Recherche Agronomique. Both are situated in south-western France.

—In Auzeville (Haute-Garonne), the sampled soil is a silt clay on an alluvial deposit (eutrochrept). Three plots, P1 to P3, which have been cropped continuously with corn for 13 yr, without any organic manure, have been sampled every 2 yr since the beginning of the experiment. Average annual yields were 8 tons of grain·ha^{-1}. After harvest, leaves and stalks were incorporated back into the soil. Before the experiment, soil had been under mixed rotations, without corn. A nearby meadow soil was sampled, and is taken as the initial δ^{13}C value due to the prior C3 vegetation.

—In Doazit (Landes), the sampled soil is a weakly weathered sandy loam on an eolian deposit (Hapludalf). The experimental plots, studied by Lubet and Juste (1979), have been under continuous corn cultivation for 23 yr, after pine-forest clearing. In a first trial, T1, leaves and stalks were incorporated back into the soil; in a second trial, T2, they have been removed for the last 17 yr. Equivalent samples in an adjacent plot under forest vegetation (*Pinus*, *Quercus* and Pteridae) were also collected. Average yields of grain were 7.5 t·ha^{-1}. No organic manure was applied. Main characteristics of the topsoils are shown in Table 1.

Processing of samples

Representative composite samples were obtained from 12 cores of 0.4 kg collected in each plot, mixed together, air-dried and crushed (<2 mm). Samples from Auzeville were then gently ground through a 200 μm sieve, in order to remove coarser plant fragments. Samples from Doazit were separated into particle size fractions. Soil was first disaggregated by mechanical shaking in water, with 5 mm dia glass balls. Sand and sand-sized plant fragments were separated at 200 and 50 μm by wet-sieving. Further dispersion of the resulting 0–50 μm suspension was obtained by stirring with sodium metaphosphate (1 g·l^{-1}).

Table 1. Main characteristics of topsoils (0–30 cm)

	Doazit		Auzeville		
Plot	T1	T2	P1	P2	P3
Clay 0.2 μm (%)	13	13	26	27	28
Silt 2–50 μm (%)	45	45	42	41	40
Sand 50–2000 μm (%)	42	42	32	32	32
pH	6.0	5.8	7.0	6.1	6.2
Total organic carbon (‰)	10.5	9.6	10.2	9.1	8.9

The fine clay fraction (0–0.2 μm) and coarse clay fraction (0.2–2 μm) were separated by centrifugation and freeze-dried. The silt fractions (2–20 and 20–50 μm) were separated by sedimentation and oven-dried at 40°C. Each separation was obtained by five successive sedimentations. Fine clays were flocculated by addition of 10 g·l^{-1} NaCl; water soluble material was discarded. All fractions were then ground by hand in a mortar.

Total carbon and nitrogen were determined with a CHN autoanalyser (Carlo Erba 1106). Carbon isotope ratios of plant and soil organic samples were measured on the CO_2 obtained by combustion under pure oxygen atmosphere at 900°C. The gas was then purified and analyzed on a mass spectrometer fitted with triple ion collectors and dual inlet system equipped for rapid switching between reference and sample (VG SIRA 9 and Finnigan Delta E). The laboratory reference has been calibrated vs PDB, using the international standard now available, NBS 19.

Analytical precision determined as the standard deviation obtained on different combustions of the same perfectly homogenized sample, is better than 0.05‰. For samples of soil organic matter, precision will depend primarily on sample heterogeneity. In the case of the plant organs analyzed, the order of magnitude of this precision is about 0.3‰. In both fields studied, we measured mean δ^{13}C values of -12.4‰ for corn leaves and stalks and -12.2‰ for roots. The value $\delta_1 = -12.3$‰ will be used in the calculation of x (equation 2).

RESULTS AND DISCUSSION

Carbon isotope ratio of initial soil organic matter under C3 vegetation

In Auzeville, the δ^{13}C value of the organic matter of soil sampled from the meadow plot, considered as reference (initial δ_0 value), was found to be -26.2‰. In Doazit, δ^{13}C of bulk forest topsoil was -26.7‰. However in this soil, small differences are observed between particle size fractions (Figs 2 and 3); deeper horizons are a little richer in ^{13}C than topsoil and clay-associated organic matter is richer in ^{13}C than bulk samples. Such small variations in isotopic composition of soil organic matter are common. Higher ^{13}C content in organo-mineral horizons than in litter and increasing δ^{13}C value with depth have been described (O'Brien and Stout, 1978). Similarly, differences between soil organic matter fractions have been reported (Nissenbaum and Shallinger, 1974). These differences generally do not exceed 1 or 2‰. Three main phenomena provide the most plausible explanation.

(i) The respiration of a substrate by micro-organisms could involve a normal isotope effect, so that respired CO_2 is depleted in ^{13}C, whereas derived microbial products become richer in ^{13}C than the substrate. Such an effect is described by Smejkal *et al.* (1971) in the assimilation of lactate by sulfate-reducing bacteria. But other authors observe no bulk enrichment of microbial products; see, e.g. Monson and Hayes (1982) in the case of *E. coli* grown on glucose.

(ii) Carbon isotope ratios of plant and micro-organisms display some intermolecular and intramolecular variations. For instances, lipid components are always depleted in ^{13}C, up to 10‰ (Galimov, 1974; Deines, 1980; Monson and Hayes, 1982), glucides and lignins show values close to the mean value of plant material while carboxyl groups are generally ^{13}C-enriched (Smith, 1972). Faster mineralization of some components could thus cause a differential isotope enrichment. From this assumption, Nissenbaum and Kaplan (1972) explain the difference observed between fulvic and humic acids of many soils, the former being always enriched in heavy carbon (about 0.9‰) as compared to humic acids, whose isotope composition is nearer to that of the vegetation growing on the soil.

The intermolecular variations can be illustrated by differences observed between organs of the same plant. Samples (5 mg) were collected from different parts of a single corn plant grown in an experimental field of I.N.R.A. (Versailles, France) and their ^{13}C content measured (Table 2). Differences between organs as high as 2.6‰ are observed (see leaves vs seeds). Heterogeneity between micro-samples of the same organ type did not exceed 0.3‰. No differences are observed between inner parts and cortex of stalks or roots and between living and dead leaves.

(iii) Local and general climatic variations with time can modify the δ^{13}C of atmospheric CO$_2$, the isotope fractionation by the vegetation or the vegetation itself (Stuiver, 1975, 1983; Smith *et al.*, 1973). Thus, old organic matter could so, in some cases, show δ^{13}C values slightly different from those of young organic matter.

In the soils studied, these variations do not exceed 1‰. It is nevertheless necessary to use, as initial δ_0 value in the calculation of x (equation 2), the δ^{13}C value of the equivalent sample or fraction obtained from the reference soil. Under this condition, the interpretation of soil ^{13}C content as resulting from a simple mixing of two sources of organic matters respectively with δ_0 and δ_1 isotope compositions, is a good approximation. The interpretation could be slightly different only in the case where, within the initial soil, stable organic matter would have had different ^{13}C/^{12}C ratios than the easily degradable organic matter.

Table 2. Isotopic composition of 5 mg samples from differents parts of a single corn plant (INRA Versailles experimental field)

Organ	Number of samples	Average δ^{13}C (‰)	Standard deviation
Leaves	6	−13.5	0.2
Sheaths	3	−12.6	0.3
Stalks	6	−11.9	0.3
Shucks	3	−11.9	0.4
Seeds (albumen)	3	−10.9	0.2
Seeds (cortex)	3	−12.1	0.1
Roots	12	−11.8	0.3

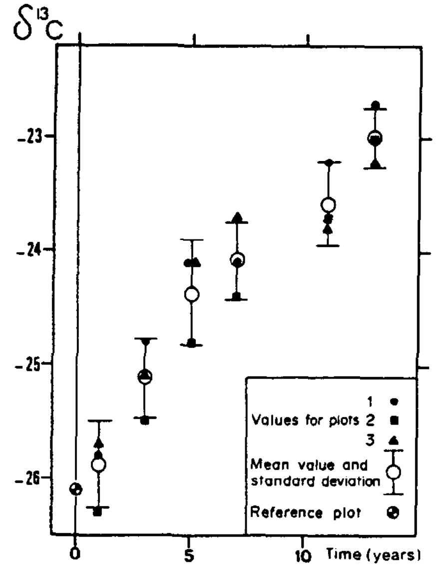


Fig. 1. δ^{13}C evolution of soil organic carbon under continuous corn cultivation (Auzeville experimental plots, topsoils 0–30 cm).

Carbon isotope composition of corn plant material

In both fields studied, the mean value of δ^{13}C for corn leaves and stalks was −12.4‰. Roots had δ^{13}C values of −12.2‰ in both cases. These values are slightly different from those obtained on the corn plant grown in other conditions (Versailles experimental field, Table 2). The mean value $\delta_1 = -12.3$‰ will thus be used in the calculation of x (equation 2).

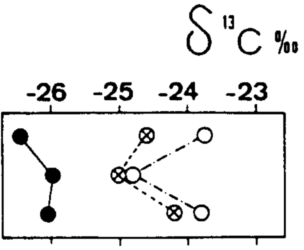
Carbon isotope composition of soil organic matter under corn cultivation

At Auzeville after 13 yr of continuous corn cultivation, δ^{13}C rose from $\delta_0 = -26.2$ to −23‰ (Fig. 1). These values mean that $x = 22\%$ of the organic carbon had turned-over since the beginning of the experiment. The annual increase in ^{13}C abundance is higher during the first years (0.35 $\delta \cdot \text{yr}^{-1}$) than during the last (0.2 $\delta \cdot \text{yr}^{-1}$). This indicates that the decay of soil organic matter cannot be described by a simple compartment model as proposed by Jenkinson (1965). The total organic carbon content being about 9 $\text{mg} \cdot \text{g}^{-1}$ soil, this enrichment of 0.2 $\delta \cdot \text{yr}^{-1}$ corresponds to an annual carbon input to the soil of 0.13 $\text{mg} \cdot \text{g}^{-1} \cdot \text{yr}^{-1}$ (50 $\text{g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$). In fact, total organic carbon content decreased by about 1 $\text{mg} \cdot \text{g}^{-1}$ soil during the experimentation from 9.5 to 8.5 $\text{mg} \cdot \text{g}^{-1}$, indicating that the amount of C previously returned to the soil was higher than that returned from corn. The total organic carbon at 13 yr is 8.5 $\text{mg} \cdot \text{g}^{-1}$. Of this, 78% is carbon from the original soil, that is 6.6 $\text{mg} \cdot \text{g}^{-1}$. The original organic carbon has reduced from 9.5 to 6.6 $\text{mg} \cdot \text{g}^{-1}$ in 13 yr. Assuming exponential decay, the turnover time is $13/\ln(6.6/9.5) = 36$ yr.

Such a value is lower than those calculated from radiocarbon dating for temperate countries [63 yr (O'Brien and Stout, 1978), 75 yr (Balesdent and Guillet, 1982)].

For the Doazit soil, two treatments were compared. In the first one (T1), corn leaves and stalks

Depth (m)	T1 trial Organic C mg.g^{-1}	T1 trial x %	T2 trial Organic C mg.g^{-1}	T2 trial x %
0-30	14.5	19	12.7	13
30-40	7.0	8	5.4	7
40-60	3.5	17	4.6	13



x = *percent of organic carbon from corn*

● *forest soil*

○ *T1 : restitution of leaves and tips*

⊗ *T2 : no restitution*

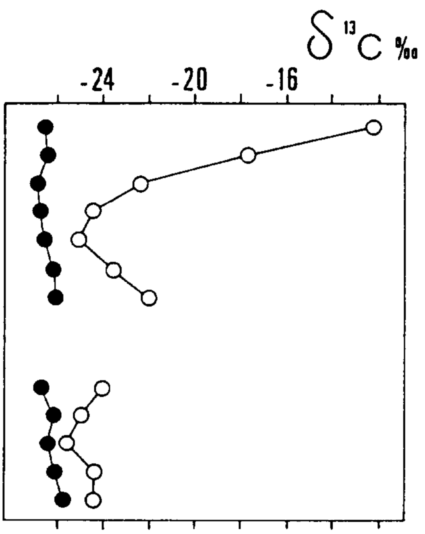
Fig. 2. Organic carbon content and $\delta^{13}C$ of 0–50 μm fractions in a soil under forest (which acts as a reference) and under soils after 23 yr continuous corn cultivation (Doazit experimental plots T1 and T2).

were incorporated back into the soil. In the other one (T2) they were removed as for forage corn cultivation. Figure 2 shows the carbon and ^{13}C content of forest soil, T1 and T2 treatments, the latter two after 23 yr of continuous corn cultivation. Results are reported for the particle size fractions 0–50 μm which contain most of the soil organic carbon, except plant fragments. Within the topsoil (0–30 cm) of T1 treatment, 19% of the carbon had turned over after the 23 yr of experimentation, corresponding to an absolute quantity of 2.8 mg·g^{-1} of the 0–50 μm fraction. This quantity, which corresponds to about 650 g·m^{-2}, is less than the one calculated for Auzeville soil, where 750 g·m^{-2} came from corn after only 13 yr of cultivation. This difference can be explained by the low clay content and the slight acidity of Doazit's soil. For the T2 treatment, the quantity of carbon from corn in the fraction 0–50 μm was only 1.7 mg·g^{-1} fraction. These inputs to the soil represent only 60% of the input in treatment T1 resulting in a higher total organic carbon content in the T1 treatment.

Topsoils (0–30 cm) and underlying horizon (30–40 cm) of Doazit forest soil and treatment T1 were fractionated into particle-size fractions (Fig. 3). For this low clay content soil, complete dispersion is obtained with glass balls in a sodium metaphosphate medium. A microscopic study shows that fractions coarser than 20 μm contain only sand (mainly quartz) and organic fragments. Small fragments are also observed in the 2–20 μm fraction. It can be deduced from the $\delta^{13}C$ values that the turnover of the coarse sand fraction (200–2000 μm) is the most rapid, but not complete, indicating the persistence of some forest fragments 23 yr after forest clearing. The turnover of the fractions 20–50 and 50–200 μm is much slower.

Organic matter associated with mineral particles

Particle size (μm)	C in % of soil C	C/N	x (%)
Level 0-30 cm			
Litter	ND	ND	100
200-2000	10	23	61
50-200	17	14	31
20-50	3	15	17
2-20	37	16	12
0.2-2	29	10.5	18
0-0.2	4	8.5	26
Level 30-40 cm			
50-200	7	16	16
20-50	3	ND	8
2-20	47	14	6
0.2-2	31	12.5	13
0-0.2	9	7.5	9



x = *percent of organic C derived from corn*

● *forest soil*

○ *corn cultivated soil (T1)*

Fig. 3. Organic carbon content, C/N ratio and $\delta^{13}C$ of particle-size fractions associated organic matter in soils after 23 yr continuous corn cultivation (Doazit experimental plot T1).

are found in the fractions 0–0.2, 0.2–2, and 2–20 μm. The last two fractions contain most of the soil organic carbon in this soil, characterized by a low fine-clay particle content. The percentage of carbon derived from corn after 23 yr increases from 12% in the fine silt (2–20 μm) fraction up to 26% in the fine clay (0–0.2 μm) fraction, which can be considered as younger. The relatively greater age of the organic matter associated with silt fractions has already been observed by radiocarbon dating (Anderson and Paul, 1984). Tiessen and Stewart (1983), who estimated the degradability of particle size associated organic matter from the carbon losses during cultivation, obtained similar results for chernozemic soils, stability increasing from sand up to fine silt and from fine clay up to fine silt. Turchenek and Oades (1979) described the fine silt fractions as rich in highly aromatic humic substances and in material of microbial origin. This fraction appears to be highly resistant to degradation. Because of the low specific surface of the mineral particles of this fraction, this organic matter must be only weakly bonded to the mineral particles.

The relative youth of the organic matter associated with fine clay particles is classically observed during incubation with ^{14}C or ^{15}N. On the other hand, radiocarbon dating indicates that these fractions could contain old organic matter, especially in non-hydrolysable material (Scharpenseel, 1977). These clay fractions can thus be considered as very heterogeneous with regard to degradability. They probably contain both components with a high turnover rate and stable organic matter.

We conclude that the cultivation of a C4 plant on soils which had never carried any such plant appears to provide a useful ^{13}C natural labelling of soil organic matter. It can be applied to the study of bulk soil organic matter dynamics and to the characterization of organic fractions. With a δ^{13}C precision of about 0.3‰, it gives an absolute precision of 2% on the proportion of "labelled" plant material incorporated to the soil, from 0 ($\delta = -27‰$) to 100% ($\delta = -12‰$).

When compared to the incorporation of ^{14}C labelled plant material, this tracer presents the advantage of allowing the observation of the organic matter produced *in situ* and allows experiments over longer periods. It can complement and emphasize data obtained by radiocarbon dating because it gives a precise measurement of the distribution of recent ages. This allows ^{14}C activity to be corrected from thermonuclear enrichment and the real mean age of soil organic matter can be deduced.

Acknowledgements—Thanks are extended to MM. C. Juste, E. Lubet and J. R. Marty for providing samples from their experimental plots. Financial assistance from the C.N.R.S. (PIREN, ATP "Matière organique dans les Sols") is gratefully acknowledged.

REFERENCES

Anderson D. W. and Paul E. A. (1984) Organo-mineral complexes and their study by radiocarbon dating. *Soil Science Society of America Journal* **48**, 298–301.

Balesdent J. and Guillet B. (1982) Les datations par le ^{14}C des matières organiques des sols. Contribution à l'étude de l'humification et du renouvellement des substances humiques. *Sciences du Sol, Bulletin AFES* **2**, 93–112.

Bender M. M. (1971) Variations in the ^{13}C/^{12}C ratios of plants in relation to the pathway of photosynthetic carbon dioxide fixation. *Phytochemistry* **10**, 1239–1244.

Cerri C., Feller C., Balesdent J., Victoria R. and Plenecassagne A. (1985) Application du traçage isotopique naturel en ^{13}C à l'étude de la dynamique de la matière organique dans les sols. *Comptes Rendus de l'Académie des Sciences de Paris* **300**, 423–428.

Deines P. (1980) The isotopic composition of reduced organic carbon. In *Handbook of Environmental Isotope Geochemistry* (P. Fritz and J. C. Fontes, Eds), Vol. 1, pp. 329-406. Elsevier, Amsterdam.

Deleens E., Lerman J. C., Nato A. and Moyse A. (1974) Carbon isotope discrimination by the carboxylating reactions in C3, C4 and CAM Plants. In *Proceedings of the Third International Congress on Photosynthesis*, Rehovot, Israel (M. Avron, Ed.), pp. 1267–1276. Elsevier, Amsterdam.

Galimov E. M. (1974) ^{13}C/^{12}C in kerogen. In *Kerogen* (B. Durand, Ed.), pp. 271–299. Edition Technip, Paris.

Jenkinson D. S. (1965) Studies on the decomposition of plant material in soil. I. Losses of carbon from ^{14}C labelled rye-grass incubated with soil in the field. *Journal of Soil Science* **16**, 104–115.

Jenkinson D. S. (1977) Studies on the decomposition of plant material in soil. V. The effect of plant cover and soil type on the loss of carbon from ^{14}C labelled ryegrass decomposition under field conditions. *Journal of Soil Science* **28**, 209–213.

Lubet E. and Juste C. (1979) Effets de l'introduction d'une prairie temporaire, d'un engrais vert et de l'exportation des résidus de récolte sur les monocultures de maïs implantées dans les sols sablo-limoneux du sud des Landes. *Comptes Rendus de l'Académie d'Agriculture, France*, Février 1979, 295–309.

Modenesi M. C., Matsui E. and Volkoff B. (1982) Relaçao ^{13}C/^{12}C nos horizontes humiferos superficials e nos horizontes escuros profundos dos solos de campo e mata da regiao de Campos do Jordao, Sao Paulo, Brasil. In *Proceedings of the Regional Colloqium on Soil Organic Matter Studies*, October 18–22/1982, Piracicaba SP, Brasil, pp. 155–161. CENA-Promocet, Piracicaba.

Monson K. D. and Hayes J. M. (1982) Carbon isotopic fractionation in the biosynthesis of bacterial fatty acids. Ozonolysis of unsaturated fatty acids as a mean of determining the intramolecular distribution of carbon isotopes. *Geochimica Cosmochimica Acta* **4**, 139–149.

Nissenbaum A. and Kaplan I. R. (1972) Chemical and isotopic evidence for the *in situ* origin of marine humic substances. *Limnology and Oceanography* **17**, 570–582.

Nissenbaum A. and Shallinger K. M. (1974) The distribution of the stable carbon isotope (^{13}C/^{12}C) in fractions of soil organic matter. *Geoderma* **11**, 137–145.

O'Brien B. J. (1984) Soil organic fluxes and turnover rates estimated from radiocarbon enrichments. *Soil Biology & Biochemistry* **16**, 115–120.

O'Brien B. J. and Stout J. D. (1978) Movement and turnover of soil organic matter as indicated by carbon isotopes measurements. *Soil Biology & Biochemistry* **10**, 309–317.

Scharpenseel H. W. (1977) The search for biologically inert and lithogenic carbon in recent soil organic matter. In *Soil Organic Matter Studies* Vol. II, SM 211/71, pp. 193–200. IAEA, Vienna.

Smejkal V., Cook F. D. and Krouse H. R. (1971) Studies of sulfur and carbon isotope fractionation with microorganisms isolated from springs of western Canada. *Geochimica Cosmochimica Acta* **35**, 787–800.

Smith B. N. (1972) Natural abundance of the stable isotopes of carbon in biological systems. *Bioscience* **22**, 226–231.

Smith B. N. and Epstein S. (1971) Two categories of $^{13}C/^{12}C$ ratios for higher plants. *Plant Physiology* **47**, 380–384.

Smith B. N., Herath H. M. W. and Chase J. B. (1973) Effect of growth temperature on carbon isotopic ratios in barley, pea and rape. *Plant & Cell Physiology* **14**, 177–182.

Stuiver M. (1975) Climate versus changes in ^{13}C content of the organic component of lake sediments during the Late Quaternary. *Quaternary Research* **5**, 251–262.

Stuiver M. (1983) Statistics of the A.D. record of climatic and carbon isotopic change. *Radiocarbon* **25**, 219–228.

Tiessen H. and Stewart J. W. B. (1983) Particle-size fractions and their use in studies of soil organic matter. II. Cultivation effects on organic matter composition in size fractions. *Soil Science Society America Journal* **47**, 509–514.

Turchenek L. W. and Oades J. M. (1979) Fractionation of organo-mineral complexes by sedimentation and density techniques. *Geoderma* **21**, 311–343.