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SOIL ORGANIC MATTER ASSIMILATION BY A GEOPHAGOUS TROPICAL EARTHWORM BASED ON $\delta^{13}C$ MEASUREMENTS[1]

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Abstract. Assimilation of soil organic matter and fresh plant debris by *Millsonia anomala*, a tropical geophagous earthworm, was investigated by measuring changes in the $^{13}C/^{12}C$ ratio of their tissues when fed on organic matter naturally labelled by ^{13}C. Individuals collected from a soil colonized by C_4 plants (C_4 soil) that were fed on C_3 soil had >50% of tissue C derived from the C_3 soil after 33 d. Assimilation of organic matter associated with different particle size fractions was investigated with individuals fed for 25 d on C_4 soil with C_4 particle size fractions substituted in turn for the equivalent C_3 soil particle fraction. A significant labelling of earthworms was observed with both the 250–2000 μm and the 0–20 μm particle size fractions. Addition of fresh C_3 plant debris to the C_4 soil also resulted in a significant change in earthworm C isotope ratio. Assimilation of fresh plant material was greater than that of soil organic matter. This shows that young *M. anomala* are able to assimilate young organic matter (fresh plant debris, coarse soil organic matter) as well as fine soil organic matter, both of which classically have been regarded as strongly resistant to decomposition in models of soil organic matter dynamics.

Key words: assimilation; C isotope ratio; fresh plant debris; geophagous tropical earthworm; particle size organic fractions; soil organic matter.

INTRODUCTION

The role and importance of invertebrates in the maintenance of soil fertility is controversial. Whether invertebrates are determinants of soil fertility or simply "noise" in the soil–plant system has been debated for decades and yet is crucially important for the future design of land use practices (Coleman et al. 1983, Anderson 1987, Lavelle et al. 1989*b*). On the one hand are soil organic matter (SOM) models that do not explicitly include fauna or root activities but seem to satisfactorily simulate SOM dynamics anyway (e.g., Van Veen et al. 1984, Andren and Paustian 1987, Jenkinson et al. 1987, Parton et al. 1987). On the other hand are microcosm studies that occasionally show dramatic effects of invertebrate addition or removal (Elliot et al. 1979, Anderson et al. 1985, Spain et al., *in press*).

Such microcosm studies showed that particular attention should be paid to earthworms that may play a determinant role on soil fertility by their burrowing activity that influences soil porosity and water infiltration (Aina 1984), their casting activity that contributes to soil aggregation (Blanchart et al. 1989), their digestion activity that regulates microbial activity (Barois and Lavelle 1986, Barois, *in press*) and accelerates the incorporation of litter into soil (Stout and Goh 1980). In field conditions, the impact of earthworms on soil fertility is expected to be dramatic in many occasions, especially as they represent frequently >50% of the total soil fauna biomass (Lee 1985).

The role of earthworms in soil food webs is especially problematic. While we know that earthworms annually ingest a large proportion of the SOM in many ecosystems (Lavelle 1978), their general importance to nutrient and energy fluxes in the system are still uncertain due to the lack of a precise knowledge of the diet of most species (Bouché and Kretzschmar 1974, Ferrière 1980, Kanyonyo and Lavelle, *in press*) and an even larger ignorance of earthworm assimilation efficiency,

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digestive mechanisms, and the origin of digestion enzymes (Van Gansen 1961, Barois and Lavelle 1986, Loquet and Vinceslas 1987, Martin et al. 1987). That known saprophagous and known geophagous invertebrates ingest similar substrates adds to the uncertainty (Swift et al. 1978, Vannier 1985). Similar problems arise in other areas of ecological research, e.g., in freshwater ecology in addressing the question of whether an invertebrate is feeding on autochthonous organic matter (i.e., organic matter produced by the aquatic ecosystem) or on allochthonous organic matter (i.e., introduced into the system). A lack of appropriate methodologies has long impeded our clearly addressing such questions; gut content analysis gives only partial answers as the digestible resources can be easily lost prior to observation.

In this paper we present a novel method based on natural ^{13}C labelling to assess the assimilation of soil organic matter by the tropical geophagous earthworm *Millsonia anomala*. Populations of this species dominate the earthworm community in savanna soils at Lamto (Ivory Coast). They annually ingest one-third of the organic reserves of a savanna soil and up to 60% of the SOM accumulated in the upper 10 cm, the most biologically active layer (Lavelle 1978). We have estimated assimilation through the changes in tissue $\delta^{13}C$ of earthworms fed with organic material with contrasting ^{13}C content. Using this technique we tested *M. anomala*'s ability to assimilate SOM from different particle size fractions of a forest soil as compared to fresh plant material at different nitrogen contents.

Materials and Methods

Plants with the C_4 photosynthetic pathway are known to discriminate against atmospheric $^{13}CO_2$ to a lesser extent than plants with the C_3 pathway (Bender 1968, Smith and Epstein 1971). The ^{13}C natural abundance, expressed as $\delta^{13}C$ ranges from ≈ -9 to $-16‰$ in C_4 plants, and ranges from -22 to $-35‰$ in C_3 plants (O'Leary 1988). The C isotope ratio of SOM is close to that of the source plant material; as a result, soil organic matter is richer in ^{13}C (i.e., the $\delta^{13}C$ ratio is less negative) when the plant material is derived from C_4 rather than C_3 plants. Thus, introduction of C_3-derived organic matter in a soil colonized by C_4 vegetation, for example, constitutes an in situ labelling of organic matter incorporated into the soil. By making use of the unique carbon isotopic composition of C_3 and C_4 plants, vegetation changes have been demonstrated in several geographic areas by $\delta^{13}C$ measurements of SOM within the soil profile (Dzurec et al. 1985, Schwartz et al. 1986). As the $\delta^{13}C$ ratio is almost linearly correlated to the ^{13}C abundance, the C isotope ratio of a C_3:C_4 mixed soil (δ_1) measures the relative contribution of C_4 (X) and C_3 ($Y = 100 - X$) derived material to SOM:

$$X = \frac{\delta_1 - \delta_2}{\delta_0 - \delta_2} \cdot 100,$$

where δ_0 is the $\delta^{13}C$ of the C_4-derived SOM and δ_2 is the $\delta^{13}C$ of the C_3-derived SOM. This approach has allowed estimation of SOM turnover in soils of subtropical (Cerri et al. 1985, Volkoff and Cerri 1987) and temperate regions (Balesdent et al. 1987, 1988).

$$\delta^{13}C = \left(\frac{{}^{13}R_{sample} - {}^{13}R_{standard}}{{}^{13}R_{standard}}\right) \cdot 1000, \text{ where } {}^{13}R = \frac{{}^{13}C}{{}^{12}C}$$

Since it has been demonstrated that the $\delta^{13}C$ value of an animal is nearly the same as the $\delta^{13}C$ of its diet (De Niro and Epstein 1978), SOM assimilation by earthworms can be calculated from changes in the $\delta^{13}C$ of their tissues when they are fed with SOM with contrasting $\delta^{13}C$. The purpose of this paper was to test the ability of earthworms to digest "labile" and "resistant" SOM. To accomplish this, we monitored, in laboratory cultures, changes of $\delta^{13}C$ of earthworms taken from a soil colonized by C_4 plants (C_4 soil) and fed with a C_3 soil or with a C_4 soil complemented with C_3 fresh plant material; in another set of experiments, we measured also changes of $\delta^{13}C$ of earthworms taken from a soil colonized by C_4 plants (C_4 soil) and fed with a C_3 soil in which one particular particle size organic fraction was removed and replaced by equivalent material from a C_3 soil.

Site characteristics

This study was carried out at Lamto, Ivory Coast (5° N, 6° W); the climate is characterized by a high mean annual temperature (28°C) and irregular annual rainfall (rainfall averages: 1300 mm/yr, with a dry season from November to March and in August) (Lecordier 1975). Most soils lie on a granitic bedrock and are classified as "sols ferrugineux tropicaux" (Riou 1974) or Ferralsol (FAO 1988), which is very similar to an Oxisol.

Vegetation is a mosaic of grass savannas and forest patches (César et Menaut 1974). Savanna, the most widespread vegetation type, is maintained by regular annual burning. *Loudetia simplex* and several species of Andropogoneae (Gramineae, all C_4 plants) largely dominate the grass cover. Some C_3 plants, such as legumes and palm trees (*Borassus aethiopum*), also grow in shrub savannas; however, these plants provide little organic matter to the soil, and the topsoil of all savannas has a $\delta^{13}C$ value of -12.3 to $-12.8‰$, typical of C_4 vegetation (Martin et al. 1990). Grass savanna soil is highly sandy and has a very low clay content ($\approx 2\%$). Water retention is low (field capacity $\approx 10\%$). This soil has a very low C content (5 mg/g soil) and the C/N of the 0–10 cm horizon is 13.9. The pH ranges from 5.5 to 6.5. Rivers are fringed with gallery forests dominated by C_3 plants, and the topsoil $\delta^{13}C$ ratio is $\approx -27.8‰$

(Martin et al. 1990). The forest soil has a loamy texture, and the field capacity is ≈20%. The topsoil has a high C content (20 mg/g soil), and the C/N ratio of the upper 10 cm is ≈10. Topsoil pH is 5.2.

General ecology of M. anomala

Earthworms present a great diversity of ecological patterns (Bouché 1977): epigeic species are small, pigmented, and live in the litter, while anecic species are larger, partly pigmented species, and live in deep soil burrows. Both groups feed on litter. Endogeic species, on the other hand, are medium sized, unpigmented, and live in temporary soil channels; they have a geophagous diet. Distribution of earthworm species within the latitude shows a weak predominance of epigeic and anecic species in temperate regions while in the humid tropics earthworm communities are largely dominated by endogeic species (Lavelle 1988).

Millsonia anomala Omodeo and Vaillaud is the most common species of the humid savannas of Central Ivory Coast. They usually inhabit the upper 15 cm of soil and are classified as endogeics (Lavelle 1981). At Lamto their mean annual fresh biomass ranges from 70 to 250 kg/ha in savannas depending upon shrub density and annual rainfall; in gallery forest soils, biomass is much lower (6 kg/ha). In grass savannas, populations may ingest annually up to 1.2 Mg/ha dry soil, 75% of which is in the upper 10 cm of soil (Lavelle 1978).

Texture of the casts produced by young *M. anomala* in laboratory conditions, as compared to the soil they feed on, has shown that animals ingest bulk soil without any selection (Martin, 1991). In similar experimental conditions, the soil used for the culture may thus be used as reference soil as it is very much representative of what the worm actually ingests. As a result the amount of C ingested by the worm during an experiment may be estimated by multiplying the amount of casts produced by the C ratio of the soil used for the culture if we assume the mass loss due to the gut transit to be negligible.

Description of experimental soils

Soils were collected from two sites at Lamto from the 0–10 cm stratum of soils; one site possessed C_4 vegetation (grass savanna), and the other possessed C_3 vegetation (gallery forest). The soils were air-dried, homogenized, and passed through a 2-mm mesh screen to remove live roots and obtain homogeneous samples.

The original savanna soil (S_{OS}) was used to estimate the growth rate of the earthworms during the experiment. The original gallery forest soil (S_{GF}) was used to estimate the overall assimilation rate of organic matter by earthworms fed on that soil.

The assimilation of particle size organic fraction was studied with savanna soil samples with one particle size organic fraction substituted by an equivalent fraction taken from the forest soil (soils referred as to S_{250GF}, S_{100GF}, S_{50GF}, S_{20GF}, and $S_{0\text{-}20GF}$; see Table 1). Those samples were prepared according to the method described by Feller (1979) to separate organic matter of the grass savanna and the forest soils into five particle size fractions (250–2000, 100–250, 50–100, 20–50, and 0–20 μm): 50 g of soil were dispersed into 200 mL of water. After a first sonication (80W for 30 min), the soil was sieved underwater at 250- and 100-μm mesh to isolate the first two fractions (250–2000 and 100–250 μm). The suspension of fine particles was then sonicated more strongly (160W, 1.5 min) to break microaggregates, and the last three fractions were isolated by wet sieving at 50- and 20-μm mesh. In size fractions >20 μm, organic matter was separated from mineral material by flotation. Four organic particle size fractions (OF) were thus obtained: 250–2000 (referred to as 250 OF in the text), 100–250 (100 OF), 50–100 (50 OF), and 20–50 μm (20 OF). The 0–20 μm fraction (0–20 OMF) is organomineral in nature. Twelve kilograms of savanna soil and 2 kg of forest soil were processed in the above-mentioned way. In 2 kg of savanna soil, the 250 OF was removed and replaced by the same amount of the corresponding fraction extracted from the forest soil. After substitution, the experimental soil (S_{250GF}) was restructured by air-drying to field capacity after water saturation. The 100–250, 50–100, and 20–50 μm organic fractions and the 0–20 μm organomineral fraction were alternately substituted in the same way to obtain the experimental soils referred to as S_{100GF}, S_{50GF}, S_{20GF}, and $S_{0\text{-}20GF}$. Two kilograms of savanna soil, submitted to equivalent physical treatment, without organic fraction substitution, were used as a control (S_{SS} = sonicated savanna soil).

Three other C_4-C_3 mixed soils were prepared to study the assimilation of fresh organic material by the earthworms (Table 1). In some savanna soil, the coarse organic fraction (250–2000 μm) was removed and replaced by an equivalent amount of C from fragments of fresh legume leaves (*Eriosema* sp., C_3 plant). This soil is referred to as S_{250Leg}. Some savanna soil was also supplemented with fresh C_3 plant fragments (wheat roots or legume leaves [*Eriosema* sp.]); these samples are referred to as S_{Wheat} and S_{Leg}, respectively. In both cases, the amount of C_3-C added to the soil was equal to 20% of the total soil, that is, the amount of C contained in the savanna soil 250 OF.

Experimental design

Soils were moistened at field capacity (i.e., 10%) and further passed through a 2-mm mesh screen; this sieving of wet soil allows measurement of soil ingestion by separating casts from the noningested soil (Lavelle 1975). Three hundred grams of wet soil were then put into plastic boxes; six replicates were prepared for each experimental soil.

Young *M. anomala* (0.1–0.2 g fresh mass) were collected in the upper 10 cm of a savanna soil. They were weighed individually, and one individual was put into each plastic box. The experiment lasted 25 d when

TABLE 1. Description of soils used to study the assimilation of soil organic matter by *Millsonia anomala.*

Soil*	Original site	Sonication	Treatments: Addition of C_3 fresh plant debris	Treatments: Substitution of a particle size organic fraction
S_{OS}	Grass savanna	no	no	no
S_{Wheat}	Grass savanna	no	20% of wheat roots fragments	no
S_{Leg}	Grass savanna	no	20% of legume leaf fragments	no
S_{SS}	Grass savanna	yes	no	no
$S_{250\ Leg}$	Grass savanna	yes	no	250–2000 μm OF by legume leaf fragments
$S_{250\ GF}$	Grass savanna	yes	no	250–2000 μm OF by 250–2000 μm OF of gallery forest soil
$S_{100\ GF}$	Grass savanna	yes	no	100–250 μm OF by 100–250 μm OF of gallery forest soil
$S_{50\ GF}$	Grass savanna	yes	no	50–100 μm OF by 50–100 μm OF of gallery forest soil
$S_{20\ GF}$	Grass savanna	yes	no	20–50 μm OF by 20–50 μm OF of gallery forest soil
$S_{0-20\ GF}$	Grass savanna	yes	no	0–20 μm OF by 0–20 μm OF of gallery forest soil

* For definition of soil types and fractions, see *Materials and methods: Description of experimental soils.*

worms were fed on savanna soils and 12 or 33 d when they were fed on forest soil. During the experiment soil temperature and moisture were kept constant (temperature = 28°C, moisture = 10%).

At the end of the experiment, earthworms were weighed to estimate the amount of new C incorporated into their body during the experiment (growth rate, A) defined as:

$$A\ (\%) = \frac{M_f - M_i}{M_f} \cdot 100, \qquad (1)$$

where M_f is the final fresh mass and M_i the initial fresh mass of individuals. Earthworms were further sacrificed by immersion for 1 s in boiling water. Gut contents were removed, and the bodies were dried at 40°C for one night and weighed to evaluate the dry mass of individuals.

Soil was air-dried; casts and noningested soil were separated by dry-sieving through a 2-mm mesh screen to determine the amount of soil ingested (B) during the experiment. The amount of C_3-C ingested by each animal was calculated by multiplying the C_3-C rate of the soil used for the culture by the amount of casts produced by the worm during the experiment. Such a calculation is relevant because *M. anomala* ingests the soil without any selection.

Earthworms were also collected from the upper 10 cm of the soil of the gallery forest and savanna; they were killed immediately and prepared as indicated previously to obtain control values of $\delta^{13}C$.

C content and $\delta^{13}C$ measurements

Carbon contents of soils and organic fractions were determined by coulometric titration after complete combustion of samples at 900°C in a pure oxygen atmosphere. Because these soils are free of carbonate, the carbon content thus measured is equal to the organic carbon content. Precision of measurements is ≈5% depending primarily on sample heterogeneity.

The ^{13}C natural abundance of earthworms, organic fractions, and soils was determined on the CO_2 gas obtained after total combustion of organic matter in a sealed quartz tube with CuO at 900°C. After breaking the tube *in vacuo,* the CO_2 evolved was purified over pure reduced CuO heated at 600°C, dried, and analyzed in an isotope ratio mass spectrometer (Finnigan Delta E and VG Sira 9) fitted with triple ion collector and dual inlet system equipped for rapid switching between reference and sample (Wedeking et al. 1983). Results are obtained in $\delta^{13}C$‰ units. Laboratory references were calibrated using NSB19; results are expressed versus the international PDB standard (Craig 1957). Analytical precision on perfectly homogenized samples is ±0.05‰.

Each treatment was replicated 6 times; mean values and standard errors of growth rate, ingestion rate, and $\delta^{13}C$ ratio were calculated. Statistical analyses consisted of ANOVA tests. The level of significance was defined as .05.

RESULTS

Soil characteristics

Distribution of C between particle size classes shows that the bulk of organic matter in both the grass savanna (S_{OS}) and gallery forest soils (S_{GF}) is accumulated in the smallest 0–20 μm organomineral fraction (Table 2). In both soils, comparable $\delta^{13}C$ values are observed within the particle size fractions. In contrast, equivalent organic fractions from the savanna and forest soils have very different carbon isotope ratios: −12.6 to −12.9‰ in the C_4 soil, as compared to −27.6 to −28.1‰ in the C_3 soil.

$\delta^{13}C$ ratio of earthworms in natural conditions

At both sites, $\delta^{13}C$ measurements of worms gave remarkably homogeneous values. $\delta^{13}C$ ratio ranged from −23.3 to −23.8‰ in forest individuals, and from −11.2 to −12.3‰ in savanna earthworms (Table 3). Mean $\delta^{13}C$ values of earthworms were always greater than soil values. The difference reached 1‰ in savanna and 4.3‰ in forest.

TABLE 2. Properties of soil used in experiment that measured assimilation of soil organic matter by the earthworm *Millsonia anomala*. Soil was from 0–10 cm depth under two vegetation types at Lamto, Ivory Coast.

Soil particle size (μm)	Relative mass (%)	C distribution (mg/g soil)	Organic fraction C/N	Organic fraction $\delta^{13}C$ (‰)
		Grass savanna		
250–2000	44.6	0.84	18.2	−12.8
100–250	32.4	0.54	15.4	−12.8
50–100	9.9	0.65	19.7	−12.7
20–50	5.3	0.87	17.3	−12.6
2–20	5.9	1.42	13.2	−12.8
0–2	1.9			
Total soil	100	4.32	16.2	−12.8
		Gallery forest		
250–2000	3.3	1.9	22.2	−27.6
100–250	4.9	0.6	15.7	−27.7
50–100	22.2	1.2	13.9	−27.8
290–50	32.4	1.6	13.2	−28.1
2–20	36.4	12.3	10.8	−27.9
0–2	11.9			
Total soil	100	17.5	12.6	−27.8

Growth rate and variation of $\delta^{13}C$ of earthworms in control conditions

The growth rate (A) of individuals fed for 25 d on the control savanna soil in the laboratory was quite variable, and ranged from 13 to 36%, with an average of 27.5% (Table 3). No significant differences in growth or soil ingestion rates (Tables 4 and 5) were observed between earthworms fed on sonicated control soil (S_{SS}) and original control soil (S_{OS}) for 25 d. However, sonication of soil resulted in a slight isotopic enrichment of the earthworms as the $\delta^{13}C$ ratio of the animals fed on the sonicated control soil (S_{SS}) decreased from −11.8 ± 0.4‰ to −12.2 ± 0.4‰ in 25 d (Tables 3 and 4).

Variation of $\delta^{13}C$ of earthworms fed on forest soil

$\delta^{13}C$ of savanna earthworms fed on forest soil decreased sharply from −11.8 ± 0.3‰ to −14.8 ± 0.3‰ after 14 d, and reached −18.4 ± 1.1‰ after 33 d of the experiment (Fig. 1).

Growth rate and labelling of earthworms fed on savanna-forest mixed soils

Earthworm growth rate did not change significantly except when the finest fractions (<50 μm) were substituted. A 25% increase was observed when the 0–20 μm organomineral fraction (0–20 OMF) was substituted, while a 39% increase was observed with the 20–50 μm organic fraction (20 OF) (Table 4). Ingestion rate of soil significantly increased (+38%) for individuals fed on $S_{0\text{-}20GF}$, and remained constant in other treatments. The $\delta^{13}C$ of earthworms decreased in all experimental soils (Table 4), but significant differences were observed only when the 250–2000 μm organic fraction (250 OF) or the 0–20 OMF of the savanna soil were replaced by equivalent forest organic fractions. In the latter case, the difference reached −1.4‰.

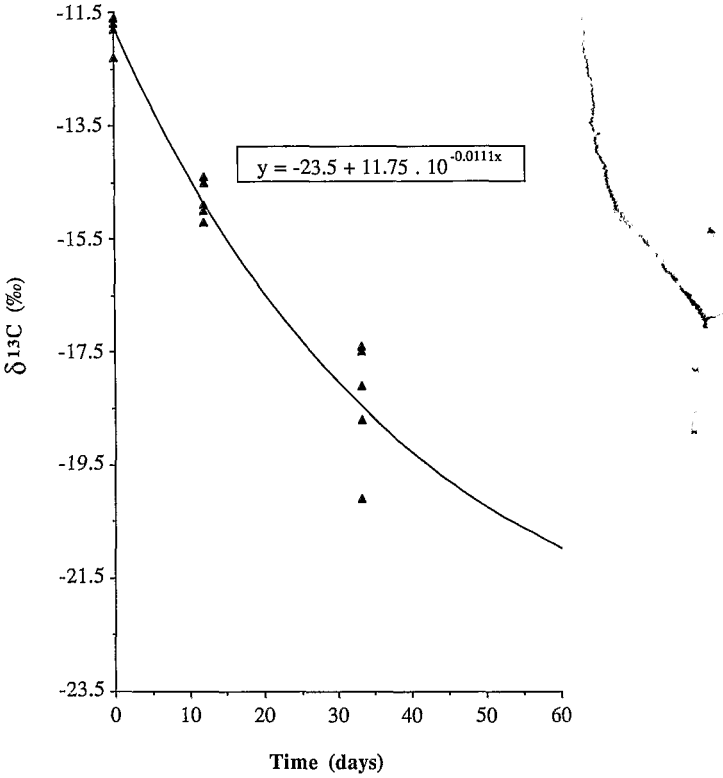

FIG. 1. Temporal changes in $\delta^{13}C$ values of earthworms in response to being switched from savanna to forest soil.

Growth rate and $\delta^{13}C$ of earthworms fed on savanna soil supplemented with fresh organic material

A significant increase in growth rate was observed when animals were fed for 25 d on C_4 soil enriched

TABLE 3. Growth rate (A), soil ingestion rate (B), and $\delta^{13}C$ ratios (in ‰) of earthworms (*Millsonia anomala*) fed on savanna or forest soils (C_4 or C_3 organic matter, respectively). $N = 6$ except for $\delta^{13}C$ ratios of soils.

Soil	Statistics*	A† (%)	B‡ (g dry soil)	$\delta^{13}C$ (‰) Earthworms	$\delta^{13}C$ (‰) Soil
Savanna soil (S_{OS})	$\bar{X}$	27.5	138	−11.8	−12.8
	σ_{n-1}	8.2	11	0.3	ND§
Forest soil (S_{GF})	$\bar{X}$	ND	ND	−23.5	−27.8
	σ_{n-1}	ND	ND	0.2	ND

* $\bar{X}$ = mean, σ_{n-1} = standard deviation.
† A = the final percentage of new C incorporated into the body of the worm in 25 d.
‡ B = the amount of soil ingested by worms in 25 d.
§ Not determined.

TABLE 4. Final characteristics of earthworms (*Millsonia anomala*) collected in a savanna soil (C_4 SOM) and further fed for 25 d on a savanna soil (C_4 SOM), one organic fraction of which has been replaced by either an equivalent fraction extracted from a forest soil (C_3 SOM) ($S_{i\ GF}$ series) or by debris of fresh legume leaves (C_3 plant) ($S_{i\ Leg}$ series), compared to savanna soil $N = 6$ in all cases. Letters on the right of the means indicate results of ANOVA (level of significance: .05). Data from the same column with different letters are significantly different.

Soil*	Statistics†	A‡ (%)	B§ (g dry soil)	C_3-C ingested (mg)	$\delta^{13}C$ of tissues (‰)	C_3-C of tissues (%)
Sonicated savanna soil	$\bar{X}$	33.1 a	134 a		−12.2 a	
(S_{SS})	σ_{n-1}	6.6	26		0.4	
$S_{250\ Leg}$	$\bar{X}$	47.9 b	133 a	107 a	−14.4 b	19.9 a
	σ_{n-1}	12.8	23	18	0.3	2.5
$S_{250\ GF}$	$\bar{X}$	37.1 a	131 a	118 a	−12.9 c	6.1 b
	σ_{n-1}	9.8	15	14	0.4	3.1
$S_{100\ GF}$	$\bar{X}$	34.0 a	110 b	60 b	−12.6 a	ND‖
	σ_{n-1}	10.2	16	11	0.5	ND
$S_{50\ GF}$	$\bar{X}$	39.6 a	139 a	90 a	−12.6 a	ND
	σ_{n-1}	18.3	31	20	0.4	ND
$S_{20\ GF}$	$\bar{X}$	46.1 b	137 a	123 a	−12.8 a	ND
	σ_{n-1}	5.5	23	21	0.8	ND
$S_{0-20\ GF}$	$\bar{X}$	41.4 b	185 c	262 c	−13.6 c	12.4 c
	σ_{n-1}	1.8	16	23	0.3	2.6

* For definitions of soil types and fractions, see *Materials and methods: Description of experimental soils.*
† $\bar{X}$ = mean, σ_{n-1} = standard deviation.
‡ A = the final percentage of new C incorporated into the body of the earthworm in 25 d.
§ B = the amount of soil ingested by worms in 25 d.
‖ Not determined.

with fresh C_3 plant material (S_{250Leg}, S_{Wheat}, and S_{Leg}) as compared to soil organic matter only (Table 5). This increase of growth rate ranged from 73 to 96% and was not significantly correlated with the nature of material added to the soil (legume or wheat). No significant differences were observed in soil ingestion rate between the control soil, S_{250Leg}, and S_{Wheat}. Ingestion of fresh plant material induced a significant decrease of the ^{13}C natural abundance of earthworms: the reduction in $\delta^{13}C$ mean value was significantly greater with legume plants (−2.4‰ for S_{Leg}, − 2.3‰ for S_{250Leg}) than wheat (−1.6‰ for S_{Wheat}).

DISCUSSION

$\delta^{13}C$ values of savanna and forest soil organic fractions

The $\delta^{13}C$ values measured in the topsoil of the grass savanna and gallery forest at Lamto are typical of C_4 and C_3 vegetation, respectively (Stout et al. 1981). In both vegetation types, ^{13}C natural abundances of organic fractions are close to the value of the whole soil. Similar data are reported by Cerri et al. (1985) for a subtropical forest topsoil and Balesdent et al. (1987) for a temperate forest topsoil.

TABLE 5. Final characteristics of earthworms (*Millsonia anomala*) collected in a savanna soil (C_4 SOM) and further fed for 25 d on a savanna soil (C_4 SOM) supplemented with fresh C_3 plant debris, compared to savanna soil. $N = 6$ in all cases. Letters on the right of the means indicate results of ANOVA (level of significance: .05). Data from the same column with different letters are significantly different.

Soil*	Statistics†	A‡ (%)	B§ (g dry soil)	C_3-C ingested (mg C)	$\delta^{13}C$ of tissues (‰)	C_3-C of tissues (%)
Original savanna soil	$\bar{X}$	27.5 a	138 a		−11.8 a	
(S_{OS})	σ_{n-1}	8.2	11		0.3	
S_{OS} + wheat	$\bar{X}$	53.9 b	139 a	129	−13.4 b	13.7 a
(S_{Wheat})	σ_{n-1}	7.1	16	15	0.3	2.2
S_{OS} + legumes	$\bar{X}$	49.8	ND‖	ND	−14.2 c	20.5 b
(S_{Leg})	σ_{n-1}	ND	ND	ND	0.5	4.0

* For definitions of soil types and fractions, see *Materials and methods: Description of experimental soils.*
† $\bar{X}$ = mean, σ_{n-1} = standard deviation.
‡ A = the final percentage of new C incorporated into the body of the earthworm in 25 d.
§ B = the amount of soil ingested by worms in 25 d.
‖ Not determined.

TABLE 6. Index of assimilation (I) of fresh legume leaves, fresh wheat roots, and the coarsest and finest particle size organic fraction of the SOM by *Millsonia anomala* ($I = \frac{|\Delta\delta^{13}C|}{\text{g } C_3\text{-C ingested}}$; $N = 6$). Statistical analysis consisted of ANOVA (level of significance: .05). Data from the same column with different letters are significantly different.

Soil*	$\frac{\lvert\Delta\delta^{13}C\rvert}{\text{g } C_3\text{-C ingested}}$	
	$\bar{X}$	σ_{n-1}†
S_{250Leg}	21.3 a	2.1
S_{Wheat}	12.7 b	3.2
S_{250FG}	5.7 c	2.4
S_{0-20FG}	5.5 c	1.4

* For definitions of soil types and fractions, see *Materials and methods: Description of experimental soils.*

† σ_{n-1} = standard deviation.

$\delta^{13}C$ ratio of savanna and forest earthworms

At Lamto, earthworms are always richer in ^{13}C than the soil they feed on. Because of the extremely small analytical error associated with the $\delta^{13}C$ value, the C isotope ratios of animals collected in stable vegetation sites can be considered an accurate value reflecting the C_3 or C_4 origin of organic matter ingested by individuals.

DeNiro and Epstein (1978) have pointed out for several species that whole-body carbon of animals is on average enriched in $\delta^{13}C$ by $\approx 1‰$ relative to the diet. This pattern may result from the biological fractionation of carbon during assimilation of SOM or the differential assimilation of heavy plant components; for example proteins, most carbohydrates, and pectins are richer in ^{13}C than cellulose, lignin, and lipids (Degens et al. 1968, Wong et al. 1975, Benner et al. 1987). It may also result from a preferential loss of ^{12}C during respiration.

However, the enrichment of 4‰ observed in earthworms relative to SOM in the forest site is particularly high and may indicate that earthworms feed occasionally on soil from the 10–25 cm stratum, the $\delta^{13}C$ of which is close to −24.5‰ (Martin et al. 1990). It may also reflect a selective feeding on the algae that occasionally grow at the soil surface and whose isotopic composition might be intermediate between those of C_3- and C_4-type plants, as are aquatic algae (Deines 1980).

Labelling of savanna earthworms fed on forest soil

The rearing of savanna earthworms on forest soil resulted in a significant decrease of their ^{13}C natural abundance, which permits the estimation of their assimilation of SOM.

The percentage of C_3 (X) and C_4 ($Y = 100 - X$) derived organic matter in earthworm tissues can be calculated by classical isotope balance:

$$X(C_3) = \frac{\delta_1 - \delta_0}{\delta_2 - \delta_0} \cdot 100, \quad (2)$$

where δ_0 is the $\delta^{13}C$ ratio of tissues produced by earthworms fed on purely C_4 organic material, δ_1 is the $\delta^{13}C$ of tissues produced by earthworms fed on C_4:C_3 mixed organic material, and δ_2 is the $\delta^{13}C$ of tissues produced by earthworms fed on purely C_3 organic material. The δ_0 value is assumed to be equal to the $\delta^{13}C$ of body tissues of earthworms fed on savanna soil ($\delta_0 = -11.8‰$ for experiments with nonsonicated soil samples, $\delta_0 = -12.2‰$ for experiments with sonicated soil samples). The δ_2 value is assumed to be equal to the $\delta^{13}C$ of body tissues of animals living in the forest soil ($\delta_2 = -23.5‰$).

The calculation showed that tissues of savanna earthworms fed with forest soil are composed of 25.8 ± 2.6% of C_3-C after 12 d and 56.1 ± 9.4% of C_3-C after 33 d. Previous studies on C turnover rate of tissues by ^{13}C or ^{14}C labelling (Tieszen et al. 1983, Bouché 1984) suggest that data should be described by a multiple negative exponential function. However, due to the lack of data, we fitted data with a simple negative exponential function that describes the turnover of the more labile carbon pool within the body of young *M. anomala* (Fig. 1); the half-life of this pool may be close to 27 d, a value much lower than that calculated by Barois et al. (1987) by labelling of *Pontoscolex corethrurus,* another tropical geophagous earthworm, with ^{15}N (half-life = 64 d).

Assimilation of particle size fractions of the soil organic matter

The significant change in $\delta^{13}C$ of the earthworms observed when the 250–2000 μm and the 0–20 μm organic fractions are labelled in the soil shows that these earthworms are able to assimilate two such different kinds of SOM as coarse organic matter (plant debris) and fine clay-associated organic matter. After 25 d of experiment, earthworm tissues contained 6.2 ± 3.1% of C_3-derived organic matter when they fed on savanna soil with a C_3 250–2000 μm organic fraction and twice as much (i.e., 12.4 ± 2.6%) with a C_3 0–20 μm organic fraction. However, the index of assimilation defined as

$$I = \frac{|\Delta\,\delta^{13}C|}{\text{g } C_3 - \text{C ingested}} \quad (3)$$

showed that the 250–2000 μm and the 0–20 μm organic fractions are assimilated with the same efficiency (Table 6).

In experiments with respective substitutions of the 100–250, 50–100, or 20–50 μm fractions, the carbon isotopic ratio of earthworms always decreased; however, observed variations were not significant as compared to control values, and there was no evidence that *M. anomala* was able to assimilate organic fractions between 20 and 250 μm. Nevertheless, data obtained from the overall set of experiments of substitution

showed that the mean final $\delta^{13}C$ value of earthworms was correlated with the amount of C_3-C in the soil (Fig. 2). As a result, the fractions between 20 and 250 μm may possibly be assimilated at the same rate as the 250–2000 and 0–20 μm OF but not induce a significant labelling of the worms in a short experiment, because the 20–250 μm fractions are not abundant.

There are no directly comparable data on assimilation of organic fractions by geophagous earthworms. Many studies report that plant debris are the major constituents of the organic material ingested by temperate earthworms and thus might be preferentially assimilated (Bouché and Kretzschmar 1974). Such conclusions have been established for geophagous earthworms, i.e., *Aporrectodea rosea* (Bolton and Phillipson 1976) as well as epigeic or anecic earthworms, i.e., *Lumbricus terrestris* (Ferrière 1980). They tend to be verified by Scharpenseel et al. (1989) in a study using the natural ^{14}C labelling of SOM by the bomb (thermonuclear explosion) carbon; the body C of earthworms living in a typic Hapludoll soil from Germany consisted of modern carbon although the soil humus had a ^{14}C age of several thousand years. On the other hand, Piearce (1972, 1978) has demonstrated that *Aporrectodea caliginosa* and *Allolobophora chlorotica,* two temperate geophagous earthworms, ingest mainly well-decomposed organic materials; however, there is no evidence that temperate geophagous earthworms are able to assimilate any kind of organic material.

It has been suggested frequently but seldom demonstrated that soil microflora might be an important component of earthworm diet. There is some evidence that fungi may be digested by *L. terrestris* (see, e.g., Cooke and Luxton 1980, Cooke 1983) as well as protozoa (Rouelle 1983). The assimilation of the fine 0–20 μm organomineral fraction by *M. anomala* in the experiments presented in this study may thus partly result from the digestion of soil microorganisms, mainly concentrated in this fraction. However, microscopic observations and microbial countings in the gut content did not give such evidence for *P. corethrurus,* a geophagous pantropical earthworm, whose ecology is close to that of *M. anomala* (Barois 1987).

Labelling of earthworms fed on soil with fresh plants: assimilation of fresh organic matter

The percentage of C_3-derived carbon (X) accumulated in earthworms during 25 d of experiment as calculated from Eq. 2 was 13.7 ± 2.2% when the worms were fed on S_{Wheat} soil sample, 19.9 ± 2.5 and 20.5 ± 4.0% when they were fed on S_{250Leg} and S_{Leg} soil samples.

The labelling of earthworms obtained in the S_{250Leg} experiment (19.9 ± 2.5%) was 3 times greater than that obtained in the S_{250GF} experiment (6.1 ± 3.1%), although the total mass of labelled carbon was quantitatively similar in both cases (20% of the soil C), as was the C content of the soil (5 mg/g soil). As *M. anomala* ingest soil without selection, this difference may be explained by a greater ability of the worm to assimilate fresh organic residues, rich in easily metabolizable water-soluble compounds; this may also result from the high nitrogen content of fresh legumes (26 mg/g) as compared with the low nitrogen content of fresh legumes (26 mg/g) as compared with the low nitrogen content of the 250 OF (2.9 mg/g).

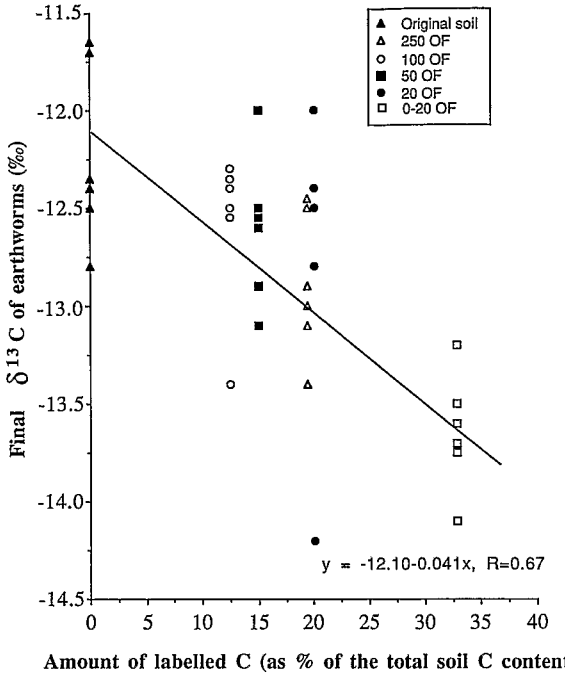

FIG. 2. Final $\delta^{13}C$ of savanna-originated earthworms fed for 25 d on a savanna soil, one particle size organic fraction of which had been replaced by an equivalent fraction from a forest soil, as a function of the amount of C present in this replacement fraction (expressed in percent of the total soil C); 250 OF: substitution of the 250–2000 μm organic fraction, 100 OF: substitution of the 50–100 μm organic fraction, etc. . . .

Ingestion of S_{Leg} and S_{Wheat} soil by savanna earthworms also resulted in a significant labelling of animals. Labelling of earthworms was lower in the S_{Wheat} experiment than in the S_{Leg} experiment, although the amount of C_3-C ingested was similar in both experiments. The assimilation of wheat root material may be less efficient than that of legume leaf material, possibly due to lower N content of wheat roots (8 mg/g) as compared with legume leaves (26 mg/g). This result is consistent with those obtained by Lavelle et al. (1989*a*): by feeding young *M. anomala* in laboratory conditions with soil added with fresh debris of *Loudetia simplex* (Gramineae), the authors observed a higher growth rate of the animals with leaf than with root debris; they concluded that fresh leaves of *L. simplex* had a higher nutritive value than roots for *M. anomala.*

Comparison of the percentage of C_3-C in earthworms fed on S_{250Leg} or S_{Leg} soil samples (19.9 ± 4.0 to 20.5

± 4.0%) to the percentage of new C incorporated into their bodies during the experiment (47.9 ± 12.8 to 49.8%) showed that the major part (≈60%) of the new C incorporated into the animals during the experiment was C_4 derived. When earthworms ingested soil supplemented with fresh legume leaves, only 26% of the C assimilated came from this legume material. These results demonstrate that earthworms simultaneously assimilated the added fresh organic matter and the endogenous SOM; however, assimilation efficiency of fresh material was 2–3 times higher than that of humified material and induced an increase of growth rate.

Ecosystem-level significance

At the ecosystem scale, ^{13}C natural labelling shows that young *M. anomala* are able to assimilate all kinds of organic matter available in soils, from fresh plant debris to coarse and fine particle size organic fractions. Organic resources accessible to these geophagous tropical earthworms are thus more diversified than those accessible to earthworms from temperate regions, which are unable to assimilate resistant organic compounds (Scharpenseel et al. 1989). The ability of geophagous tropical earthworms to digest a wide range of SOM types may result from their mutualistic relationship with soil microflora present in their digestive system (Barois and Lavelle 1986, Martin et al. 1987, Martin 1988, Barois, *in press*).

The ability of *M. anomala* to equally digest all sorts of SOM irrespective of age classes leads to questions about the concept of resistant organic matter in soil. In humid savannas of Lamto, the amount of soil ingested annually by geophagous earthworms equals as much as 1.2 Mg/ha and 60% of SOM of the upper 10 cm (Lavelle 1978). They are therefore likely to have a marked effect on SOM dynamics and soil fertility through their digestive activity. They may especially accelerate the turnover rate of the clay-mineral associated organic pool, classically regarded as resistant to decomposition in models of SOM dynamics (see, e.g., Van Veen et al. 1984, Parton et al. 1987). The ability of *M. anomala* to digest stable SOM with low C/N results in a significant release of mineral nitrogen that is thus made available to plants, *M. anomala* may produce annually in their casts 12–17 kg/ha of mineral nitrogen (S. Martin and P. Lavelle, *unpublished data*), 60% of which would originate from the clay-associated organic fraction.

We may expect from the ability of *M. anomala* to enhance the mineralization of the clay-mineral associated organic pool, which represents >60% of the SOM, a rapid loss of the soil humic pool in Lamto savannas. However, the increase of C mineralization of SOM during the gut transit seems to be balanced by an effect of SOM protection operating in the casts over a large scale of time: as a matter of fact, experiments of cast incubations in laboratory conditions demonstrated that the annual mineralization rate of SOM included in *M. anomala* casts was 3 times lower than that of SOM included in control soil (Martin 1991). If the effect of SOM protection in *M. anomala* casts occurs in field conditions, it may thus balance the effect of mineralization due to digestion.

This study demonstrates that in Lamto savannas, *M. anomala* populations participate in a significant way in the management of the humic pool of the soil. The introduction of earthworm effects into models of SOM dynamics (Parton et al. 1987) should thus be envisaged in that situation. The resulting model would provide a suitable tool (1) to quantify the impact of geophagous earthworm populations in this ecosystem and (2) to evaluate in what extent the exclusion of earthworms following forest clearance and further cultivation (Lavelle and Pashanasi 1989) may be responsible for the rapid loss of soil C and soil fertility that are commonly observed in the humid tropics (Lal 1986).

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