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C AND N TRANSFER IN SOIL WITH OR WITHOUT EARTHWORMS FED WITH ^{14}C- AND ^{15}N-LABELLED WHEAT STRAW

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Summary—The effect of earthworms (*Nicodrilus giardi giardi*) and ^{14}C- and ^{15}N-labelled plant (*Triticum aestivum*) litter on C and N transfer was studied during 31 days of incubation under controlled conditions. Respiration, food consumption by earthworms, changes in their biomass and casting production were measured. After 31 days earthworm biomass declined by 38.0% although the daily litter consumption had increased by 32% between day 10 and 31. Ingestion of C and N originating from the soil native organic matter was about 6 and 11 times larger than the ingested litter C and N respectively. Nearly all the soil C and N was rejected in casts. Incorporation of ^{14}C- and ^{15}N-labelled litter into earthworms reached 1.6 and 9.4% respectively after 31 days. This increase of the C and N from litter showed that the litter from wheat straw became more palatable after contamination by soil microorganisms. A model of microorganism and earthworm effects on litter and native soil organic matter is presented, based on the CO_2 released by different microcosms [soil (S); soil + litter (SL); soil + earthworms (SE) and soil + earthworms + litter (SEL)].

INTRODUCTION

The importance of earthworms for increasing plant productivity is well known (Stockdill and Cossens, 1969; Edwards and Lofty, 1972, 1978). Likewise, earthworms have been shown to enhance the nitrogen and phosphorus cycles (Sharpley *et al.*, 1979; Syers *et al.*, 1979; Cortez and Bouché, 1987). Earthworms also improve soil by increasing soil stability and soil infiltration rates (Slater and Hopp, 1947; Satchell 1958; Ehlers, 1975). However, less attention has been paid to the interaction and transfer of C and N derived from organic matter (soil and litter) towards other compartments as earthworms, surface and subsurface casts and CO_2 (Fig. 1). Consumption of litter and soil organic matter is related to litter palatability (Satchell and Lowe, 1967; Cooke and Luxton, 1980; Hartenstein, 1982; Cooke, 1983; Cortez and Hameed, 1988). Field studies with ^{15}N-labelled earthworms showed an important flow of N from earthworms to plants (Bouché and Ferrière, 1986). Laboratory studies with earthworms, fed with different kinds of litter, would allow estimation of the amounts of C and N, derived from litter and soil native organic matter, which will be mineralized and thus available for plants.

Our objectives were (1) to estimate the influence of palatibility of litter on the metabolism of earth-

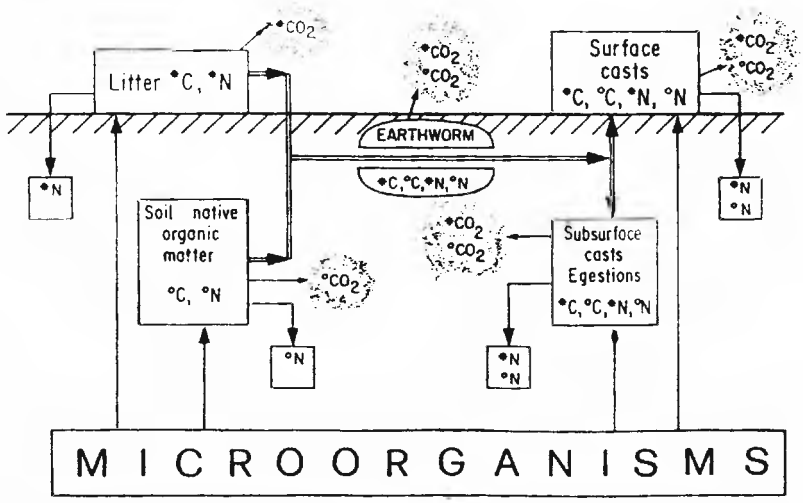

Fig. 1. Role of earthworms in transferring carbon and nitrogen derived from ^{14}C- and ^{15}N-labelled wheat straw litter (*C, *N) and from soil native organic matter (°C, °N).

Table 1. Cumulative values of total C-CO_2, *C-CO_2 from ^{14}C- and ^{15}N-labelled wheat straw litter and C-CO_2 from soil native organic matter released by the different microcosms. ^{14}C-SA represents the specific activity of C-CO_2. (1) data expressed in mg C 100 g^{-1} dry soil and (2) data expressed in Bq mg^{-1} C

Incubation time (days)	Soil (S) Total C-CO_2 (1)	Soil + EW (SE) Total C-CO_2 (1)	Soil + litter (SL)						Soil + earthworms + litter (SEL)					
			Total C-CO_2 (1)	*C-CO_2 (1)	C-CO_2 (1)	^{14}C-SA CO_2 (2)	*C_tC (%)	Total C-CO_2 (1)	*C-CO_2 (1)	C-CO_2 (1)	^{14}C-SA CO_2 (2)	*C_tC (%)
1	0.30	0.78	0.25	0.02	0.23	194	8.0	1.00	0.02	0.98	45	2.0
2	0.66	1.91	0.51	0.06	0.45	289	11.8	2.02	0.07	1.95	98	3.5
3	0.99	2.52	0.85	0.15	0.70	475	17.6	3.06	0.20	2.86	177	6.5
4	1.33	3.33	1.25	0.24	1.01	512	19.2	4.06	0.34	3.72	225	8.4
6	1.67	4.28	2.15	0.48	1.67	595	22.3	5.41	0.74	4.67	364	13.7
9	2.09	5.69	3.07	1.06	2.01	925	34.6	7.85	2.31	5.54	787	29.4
11	2.34	6.79	3.77	1.50	2.27	1066	39.8	10.01	4.30	5.71	1150	43.0
14	2.75	8.21	4.76	2.66	2.10	1494	55.9	13.18	6.91	6.27	1404	52.4
15	2.86	8.69	5.14	2.82	2.32	1468	54.9	14.16	7.80	6.36	1475	55.1
17	3.01	9.64	5.51	3.10	2.41	1507	56.3	15.89	9.98	5.91	1682	62.8
20	3.44	11.00	6.17	4.39	1.78	1904	71.2	18.00	12.41	5.59	1846	68.9
21	3.55	11.41	6.33	4.50	1.83	1904	71.1	18.98	13.21	5.77	1864	69.6
23	3.74	12.32	6.70	5.15	1.55	2056	76.9	21.73	13.98	7.75	1722	64.3
25	3.98	13.11	7.10	5.39	1.71	2034	75.9	23.54	15.34	8.20	1745	65.2
28	4.27	14.08	7.58	5.63	1.95	1989	74.3	25.78	17.33	8.45	1800	67.2
31	4.46	14.60	7.91	5.77	2.14	1954	72.9	27.53	18.56	8.97	1805	67.4

worms, (2) to investigate the relationships between the consumption of litter and soil native organic matter and (3) to follow the distribution of C and N into the various compartments of a proposed model (Fig. 1). The experiments used litter with a high C/N ratio (30.5).

MATERIALS AND METHODS

The following abbreviations are used: ^{14}C-SA = ^{14}C-specific activity; ^{15}N-E% = atom % excess ^{15}N; C and N = native C and N; *C and *N = C and N from labelled plant litter; C_t and N_t = Total C and N.

Soil samples

The soil was collected from a deep calcareous alluvial grassland soil under humid mediterranean climatic conditions (clay 21%; silt 55%; sand 24%; C 1.3%; N 0.128%; C/N 10.2; pH H_2O 8.4).

^{14}C- and ^{15}N-labelled plant litter

^{14}C and ^{15}N wheat plants (*Triticum aestivum*) were labelled as described by Cortez *et al.* (1985). The analytical characteristics of the wheat straw material, used as food for earthworms, were: *C 44.2%; *N 1.45%; *C/*N 30.5; ^{14}C-SA 2.67 kBq g^{-1} C; ^{15}N-E% 6.57%).

Earthworms

Adult earthworms (*Nicodrilus giardi giardi*) were collected in the field by the formalin method (Raw, 1959), washed with H_2O, placed into the soil at 14 C and pF 3 and fed for 3 or 4 weeks. By then, the earthworms were fully active.

Incubation procedure

The air-dry soil was sieved (2 mm), homogenized and moistened to pF 3. Twenty-four units of the moist soil (850 g air-dry weight equivalent) were put into 24 hermetically sealed 1 l. vessels. Treatments used were:

(1) 15 replicates with soil, ^{14}C- and ^{15}N-labelled wheat straw litter and 2 earthworms (SEL)

(2) 3 replicates with soil and ^{14}C- and ^{15}N-labelled wheat straw litter (SL)

(3) 3 replicates with soil and 2 earthworms (SE)

(4) 3 replicates with soil only (S).

Each vessel contained a flask with 20 ml 0.5 M NaOH to absorb CO_2. The flasks were changed every 2 or 3 days. The vessels were stored in darkness at 14°C for 31 days. During the incubation period soil moisture was kept at pF 3.

Surface casts were collected on days 3, 6, 10, 22 and 31. They were oven-dried at 70°C, weighed and stored for analysis.

Analytical methods

Total N was determined by the Kjeldahl method. Inorganic N (exchangeable NH_4^+-N, NO_3^--N) was extracted from soil by shaking with 0.5 M K_2SO_4 (w/v 3:1) for 30 min. NH_4^+ and NO_3^- were determined by steam distillation. After titration, all ^{15}N-labelled distillates (total N and inorganic N) were acidified to pH 3.0 to prevent loss of NH_3 and evaporated to dryness (70 C) on a hot plate. N_2 gas was prepared from the resulting dry NH_4 salts by oxidation with lithium hypobromite (Rittenberg *et al.*, 1948). N_2 enrichments were determined by optical spectrometry (Guiraud and Fardeau, 1980). Isotopic composition was determined by dry and wet combustion (Bottner and Warembourg, 1976) and ^{14}C by scintillation counting after collecting the CO_2 in ethanolamine and 2-methoxyethanol (v/v 1:3). Respired $^{14}CO_2$ absorbed in the alkali was measured by adding 1 ml of the 0.5 M NaOH to 10 ml of "299TH emulsion scintillator" purchased from Packard, and estimated by ^{14}C scintillation counting.

RESULTS AND DISCUSSION

C-CO_2 release

The described procedures allowed the study of the decomposition of soil native unlabelled C and N from organic matter (°C, °N) and of C and N from litter (*C, *N). Soil microbial respiration was expressed by CO_2 release from S, microbial mineralization of litter

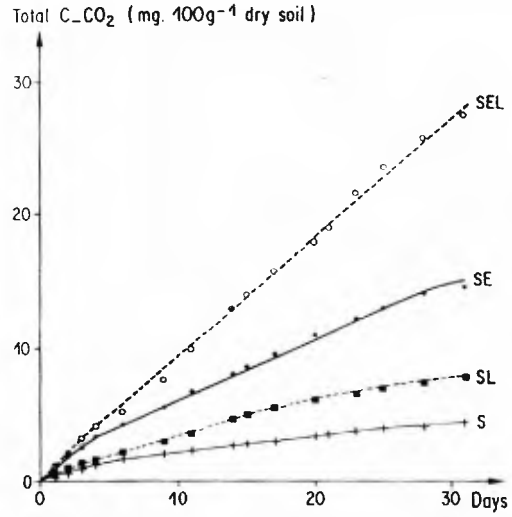

Fig. 2. Cumulative values of total CO_2 released from soil alone (S), soil + earthworms (SE), soil + plant litter (SL) and soil + earthworms + plant litter (SEL).

by CO_2 release from SL, symbiotic relationships between earthworms and microorganisms by CO_2 release from SE and the simultaneous effects of all the compartments of the system by CO_2 release from SEL. Total C-CO_2 release increased in the order S, SL, SE and SEL in the ratios 1, 1.77, 3.27 and 6.17 (Table 1). The respiration curves were nearly linear. This shows the uniformity of the respiration of the different microcosms during the incubation (Fig. 2). The addition of earthworms to S and SL to get SE and SEL increased the total CO_2 of a ratio over 3 (3.27 and 3.48). S released 4.46 mg C 100 g^{-1} dry soil day^{-1}, i.e. 0.144 mg C 100 g^{-1} dry soil day^{-1}. The addition of litter to S and SE to obtain SL and SEL respectively decreased the mineralization of soil native C (Fig. 3). After 12 days, an inhibitory litter effect on soil native C was observed (0.069 mg C 100 g^{-1} dry soil day^{-1} for SL and 0.289 mg C 100 g $^{-1}$ day^{-1} for SEL). Litter reduced on average CO_2 release by

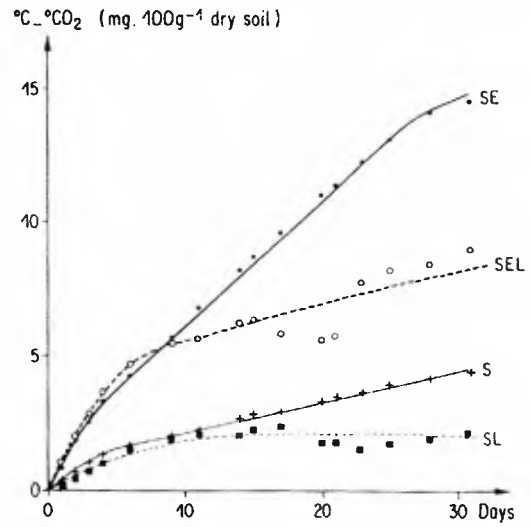

Fig. 3. Effects of plant litter addition on C-CO_2 released from soil native C (C-CO_2).

Table 2. Change in earthworms biomass, ^{14}C- and ^{15}N-labelled wheat straw litter consumption and surface casts rejected during the incubation

Incubation time (days)	Change in earthworms biomass (%)	Plant litter consumption (mg g^{-1} live worm day^{-1})	Surface casts (mg g^{-1} live worm day^{-1})
3	+9.0	7.1	30.1
6	−5.0	6.7	65.8
10	−15.2	4.8	21.2
22	−30.6	6.2	47.8
31	−38.0	7.1	97.7

50%. We cannot explain this result. It is unlikely that toxic compounds of the straw litter affected the soil microorganisms (Benoit *et al.*, 1968) or earthworms (Brattsten, 1979; Hartenstein, 1982). The earthworms added to SL to obtain SEL increased total CO_2 release, thus stimulating the mineralization of soil organic matter. $^{14}CO_2$-SA of SEL was generally higher than $^{14}CO_2$-SA of SL (Table 1). These specific activities reached constant values after 20 days which indicated a balance between the two C sources (C and *C). This balance reflects the litter quality. Cortez and Hameed (1988) have shown schematically the effect of earthworms and microorganisms on litter and soil. They found that the earthworms consumed more soil when litter was less palatable.

Biomass changes

The average earthworm biomass in SEL microcosms increased 9% of the initial live worm weight after 3 days of incubation, and decreased then regularly to a weight loss of 38% after 31 days (Table 2). These results agree with those reported by Shipitalo *et al.* (1988) who found that *Lumbricus terrestris* and *Lumbricus rubellus*, fed with different diets, changed in worm weight from a 100% gain to a 41% loss.

Litter consumption

The daily consumption of wheat straw litter varied during the incubation. The wheat straw consumption data were very close to those reported by Shipitalo *et al.* (1988) (from 6 to 13 mg g^{-1} live worm day^{-1} at 15 C depending upon the food quality). These values were also comparable, according to a $Q_{10} = 2$, to those reported by Needham (1957) who indicated a daily consumption of 27 mg g^{-1} live worm day^{-1} at 21 C for *Lumbricus terrestris*. These results demonstrate the effect of temperature on litter consumption. For *Nicodrilus giardi giardi* litter consumption decreased until day 10 and then increased up to day 31 (Table 2). However, the removal of litter from the soil surface does not imply rapid assimilation into worm tissues because some earthworm species consume microorganism-contaminated and partially decomposed litter.

Cast production and composition

From days 3 to 31 the *C and *N in casts increased (Table 3). When calculating cast production in well-defined limits (Table 4), *C and *N from litter increased in casts too despite a decrease at the end of incubation. At that time, the *C/*N ratio was 13.6 while the one of the litter was 30.5. The same pattern was reported by Bouché *et al.* (1983) who obtained a cast C/N ratio of 20 with earthworms fed with beech

Table 3. Distribution of carbon and nitrogen derived from ^{14}C- and ^{15}N-labelled wheat straw litter (*C, *N) and from soil native organic matter (C, N) in surface casts. Data (mean ± SE) followed by the same letter in the same column do not differ significantly (3 replicates; $P < 0.05$)

Incubation time (days)	C [mg g^{-1} dry wt (± SE)]	N [mg g^{-1} dry wt (± SE)]	*C [mg g^{-1} dry wt (± SE)]	*N [mg g^{-1} dry wt (± SE)]	C/N	*C/*N	*C/C (%)	*N/N (%)	Total cast production (mg)
3	15.60 ± 0.8^a	2.31 ± 0.2^a	0.62 ± 0.05^a	0.021 ± 0.005^a	6.75	29.52	6.5	0.9	754
6	12.59 ± 0.5^b	1.65 ± 0.1^b	0.25 ± 0.01^b	0.019 ± 0.008^a	7.63	13.16	1.9	1.1	2247
10	13.23 ± 0.3^b	1.35 ± 0.1^c	0.70 ± 0.05^a	0.049 ± 0.008^b	9.80	14.29	5.0	3.5	2803
22	12.68 ± 0.6^b	1.27 ± 0.1^c	1.36 ± 0.1^c	0.095 ± 0.01^c	9.98	14.31	9.7	7.0	6024
31	14.44 ± 0.4^a	0.99 ± 0.1^d	1.67 ± 0.1^d	0.107 ± 0.01^c	14.59	15.61	10.4	9.8	10.819

litter (C/N 45). The C/N ratio of the organic matter resulting from the decomposition of wheat straw was always higher in soil than in surface casts. This is an indication of the better mineralization in surface than in subsurface casts (Table 4). *C and *N found in soil were derived from litter ingested and buried in the soil by earthworms. Consequently we assumed that *C and *N found in soil represented subsurface casts and their associated microflora. Thus, the subsurface casts *N/surface casts *N ratio could be an indicator for the ratio of subsurface to the surface casting. In our experimental conditions this ratio was 13.6.

Food change of earthworms involves a modification of cast composition. So, the C/N ratio increased during the incubation. This change, essentially due to the N decrease in casts (Table 3), indicated that, during the first days of incubation, the earthworms consumed food with high N content because the wheat straw litter became more palatable. The earthworms ingested and buried more litter but they did not assimilate it.

Casting production depends on favourable climatic conditions and on earthworm diet. So, if they do not find the elements required for their metabolism, earthworms tend to explore more soil (Darwin, 1881; Abbott and Parker, 1981; Lee, 1985) and therefore produce more casts.

*Distribution of *C, *N, C and N in the different compartments*

The distribution of *C, *N, C and N in CO_2, earthworms, surface and subsurface casts after 31 days of incubation and their flow through the different compartments are shown in Fig. 4(a), (b). The *C, *N, C and N coming from the wheat straw litter and from the soil native organic matter have been measured in CO_2, surface casts and earthworms (the negative data in earthworms C and N indicated a weight loss after 31 days. In the same way, this weight loss corresponded to an output C and N flow from the earthworms lower than the input). Furthermore, we assumed that soil *C and *N corresponded to *C and *N of subsurface casts and that C/*C and N/*N ratios were similar in surface and in subsurface casts. Finally, we have calculated (1) C and N of subsurface casting and (2) C and N of soil organic matter ingested by earthworms [Fig. 4(a)].

Nicodrilus giardi giardi consumed about 5.8 times more C from soil organic matter than *C from litter. Only 2.3% of ingested C and 29.3% of ingested *C were released as CO_2. Nearly all C from soil native organic matter was excreted in subsurface casts (93%) while in surface casts the rejected C represented only 4.7% of ingested native C. 65.6% of *C and 83.3% of *N from litter was excreted in subsurface casts and 3.3% of *C and 6.1% of *N in surface casts. These data demonstrate the poor assimilation of litter by earthworms and the low C assimilation efficiency. The C/*C and N *N ratios in casts would be certainly lower if the soil used in this experiment had received a higher organic matter content. *C/C$_t$ and *N/N$_t$ ratios in surface casts increased respectively from 1.9 to 10.4% and from 0.9 to 9.8% (Table 3). These ratios showed that the earthworms incorporated more litter *C and *N at the end of the incubation. *C and *N incorporated in worms increased by 6.5 and 28.2% day^{-1} from day 22 to 31 while this increase amounted only to 11.3% and 34.3% day^{-1} during the first 22 days (Table 5). Nevertheless, after 31 days, the *C in worms represented 1.6% of the total ingested *C and the *N 9.4% of the total ingested *N [Fig. 4(b)]. All these data indicate that the assimilation efficiency of *C and *N was low but also that the assimilation rate increased with duration of incubation. After 22 days the soil microflora began to decompose the buried litter making it more palatable, thus increasing its properties for assimilation.

Model of microorganisms and earthworms effect on litter and soil organic matter

Our experimental results, based on CO_2 released by the different microcosms (S, SL, SF, SEL), allow development of a conceptual model of the action of

Table 4. Distribution of carbon and nitrogen derived from ^{14}C- and ^{15}N-labelled wheat straw litter (*C, *N) and from soil native organic matter (C, N) calculating cast production in well-defined limits. Comparison of *C *N ratios in soil and casts. Data (mean ± SE) followed by the same letter in the same column do not differ significantly (3 replicates; $P < 0.05$)

Period of cast production (days)	Surface cast weight (mg)	*C [mg g^{-1} dry wt (± SE)]	*N [mg g^{-1} dry wt (± SE)]	Cast *C *N	Soil *C *N
0–3	764	0.62 ± 0.05^a	0.021 ± 0.005^a	29.5	29.3
3–6	1483	1.14 ± 0.1^b	0.068 ± 0.005^b	16.8	24.9
6–10	556	2.34 ± 0.2^c	0.169 ± 0.01^c	13.8	27.0
10–22	3221	2.59 ± 0.2^c	0.138 ± 0.01^d	18.8	20.8
22–31	4795	1.64 ± 0.1^d	0.121 ± 0.01^d	13.6	23.7

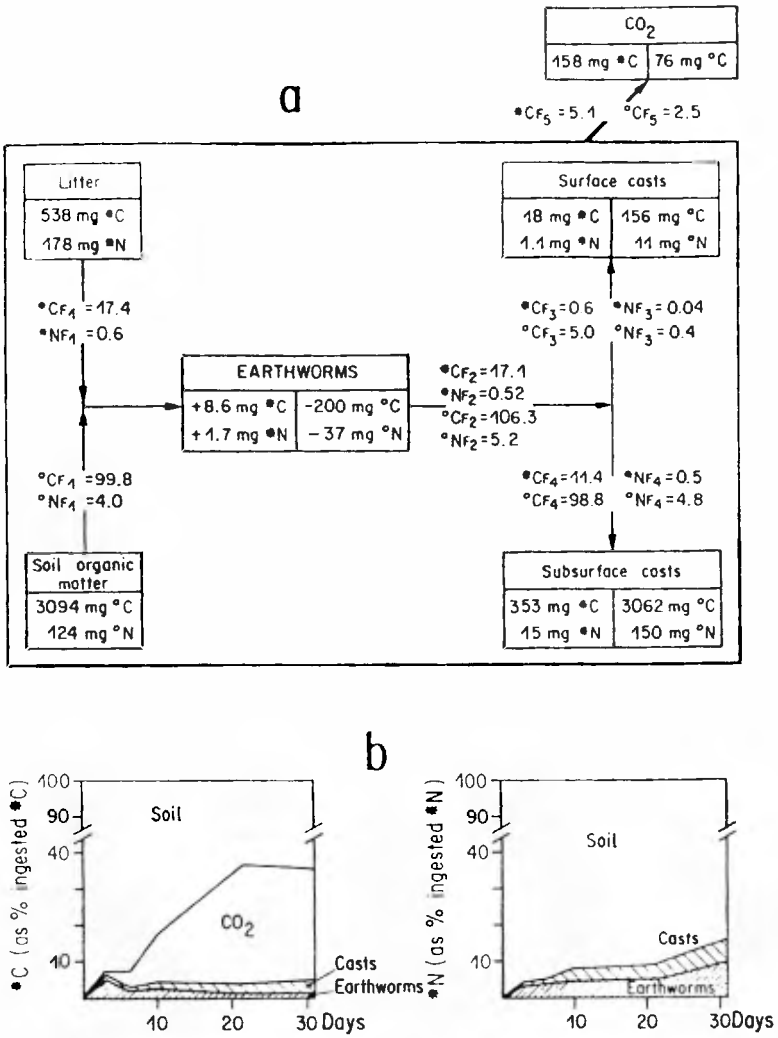

Fig. 4. (a) Quantitative estimation of carbon and nitrogen coming from ^{14}C- and ^{15}N-labelled wheat straw litter (*C, *N) and from soil native organic matter (°C, °N) in the different compartments of the model at day 31. $*C_F$, $*N_F$, $°C_F$ and $°N_F$ represent the mean of the *C, *N, °C and °N flows (as mg day^{-1}) through the different compartments. (b) Distribution of carbon and nitrogen coming from ^{14}C- and ^{15}N-labelled wheat straw litter (*C, *N) in the different compartments of the model at day 31 (expressed in % ingested *C and *N).

microorganisms and earthworms on litter and soil organic matter.

We assume that C-CO_2 released by the SEL microcosms reflected the respiration of the (1) soil microflora, (2) earthworms and (3) the litter decomposition by soil microorganisms and earthworms. The results are shown in Fig. 5. If SEL was considered as the most complete system (100%), S represented the respiration of soil microorganisms, (SL − S) the effect of soil microflora on litter, X = (SEL − SE) − (SL − S) the effect of earthworms on litter and (SEL − X) the effect of earthworms on soil organic matter. During the incubation, the effect of the soil microflora on litter was almost constant while the action of earthworms on litter increased. These results suggested that the litter became more

Table 5. Incorporation in earthworms of carbon and nitrogen derived from ^{14}C- and ^{15}N-labelled wheat straw litter (*C, *N) and from soil native organic matter (°C, °N). Data (mean ± SE) followed by the same letter in the same column do not differ significantly (3 replicates; $P < 0.05$)

Incubation time (days)	Total [mg g^{-1} live worm (± SE)]	*C [mg g^{-1} live worm (± SE)]	Total *N [mg g^{-1} live worm (± SE)]	*N [mg g^{-1} live worm (± SE)]	*C/C (‰)	*N/N (%)
3	443 ± 15^a	1.76 ± 0.05^a	81.7 ± 4^a	0.06 ± 0.01^a	0.40	0.07
6	431 ± 17^a	2.77 ± 0.1^b	85.2 ± 6^a	0.13 ± 0.01^b	0.64	0.15
10	421 ± 14^a	2.79 ± 0.1^b	78.8 ± 3^a	0.19 ± 0.01^c	0.66	0.25
22	517 ± 21^b	4.27 ± 0.3^c	100.9 ± 8^b	0.41 ± 0.05^d	0.83	0.41
31	492 ± 19^c	8.60 ± 0.5^d	94.4 ± 6^{ab}	1.68 ± 0.1^e	1.75	1.78

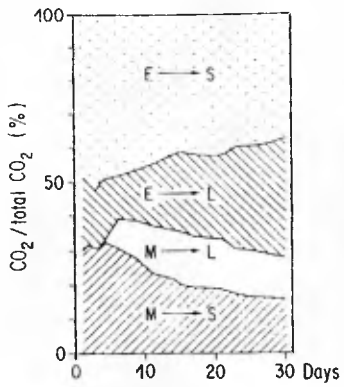

Fig. 5. Effects of microorganisms and earthworms on ^{14}C- and ^{15}N-labelled wheat straw litter and soil native organic matter, calculated by comparison of CO_2 released from the different microcosms. M→S represents the effect of microorganisms on soil, M→L of microorganisms on plant litter, E→L of earthworms on plant litter and E→S of earthworms on soil.

palatable for earthworms during incubation in the soil. It follows that the action of earthworms on soil decreased during this time. This representation is schematic because we did not consider that the litter buried in the soil tended to increase the soil microbial activity and so, we underestimated the CO_2 release from SL. However, in a study on consumption of various litter by *Lumbricus terrestris* (Cortez and Hameed, 1988), it was shown that this model could provide some insight into the behaviour of microorganisms and of the microorganism–earthworm association on different kinds of litter and on soil organic matter.

CONCLUSION

Litter quality played an important rôle in the mineralization of C and N derived from litter and soil organic matter and on the distribution of these elements in the different compartments of the model. These results agree with those of van Rhee (1963), Satchell and Lowe (1967), Edwards and Heath (1975), Brattsten (1979), Hartenstein (1979) and Cortez and Hameed (1988) who showed the relationships between chemical composition of litter and food preference of earthworms. In our experiment earthworms played a rather mechanical rôle (increase of cast production, high ingestion of soil native C and N) with their own resources. In a soil of low organic matter content, non microorganism-contaminated wheat straw litter was not an adequate or palatable food for *Nicodrilus giardi giardi*. This conclusion is based on three observations:

(1) The earthworms lost weight during the incubation, an indication of the limited assimilation of the ingested litter (litter C and N incorporated in earthworms represented 1.6 and 9.4% of ingested C and N).

(2) The earthworms ingested an important part of soil C and N, but did not assimilate them. Soil C was mostly rejected in casts.

(3) The earthworms buried litter in the soil and let it decompose before consumption. This observation was reported by Wright (1972), Cooke and Luxton (1980) and Cooke (1983). Furthermore, Abbott and Parker (1981) indicated that litter consumption was related to its nitrogen availability. Wheat straw litter had a C/N ratio of 30.5, certainly very little available nitrogen for earthworms.

In the field, earthworms consume different kinds of litter. In order to apply to field conditions the effect of microorganism–earthworm association on the mineralization of litter and the nitrogen availability for plants, we are now studying the distribution and transfer of C and particularly of N by earthworms from litter at different stages of decomposition.

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