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Decomposition of mediterranean leaf litters by *Nicodrilus meridionalis* (Lumbricidae) in laboratory and field experiments

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Abstract

The decomposition and palatability of forest leaf litters by an earthworm and soil micro-organisms were measured in laboratory and field conditions.The palatability of fresh and composted mediterranean leaf litters (*Quercus petraea* L.; *Q. ilex* L.; *Castanea sativa* Mill and *Fagus sylvatica* L.) for *Nicodrilus meridionalis* was studied in the laboratory for 31 d with a technique based on the CO_2 released by different microcosms [soil (S); soil + litter (SL); soil + earthworm (SE); soil + earthworm + litter (SEL)]. The composting of the litters increased their palatability and enhanced earthworm biomass (by 3.5–14.9% depending on the litter). In contrast feeding fresh litters resulted in a loss of earthworm biomass (by 13.2 and 14.2% for *F. sylvatica* and *Q. ilex*, respectively) and a small increase in earthworm biomass for *C. sativa* and *Q. petraea* (by 10.6 and 2.8%). The earthworm biomass was highly correlated with leaf litter quality and particularly, litter N. The decomposition rates of these leaf litters in microcosms and in the field were highly correlated. Our studies may provide a model for a quick estimate of the decomposability and palatability of different kinds of leaf litters for earthworms.

Keywords: Earthworms; *Nicodrilus meridionalis*; Leaf litter decomposition; Palatability; C and N mineralisation; Microbial leaf decomposers

1. Introduction

Many studies have been conducted on the palatability of litters for earthworms. Attempts have been made to produce correlations between physical (e.g. roughness; Satchell and Lowe, 1967) or chemical properties (N content; Abbott and Parker, 1981; Cortez and Hameed, 1988; content of phenolic compounds; Brattsen, 1979) of leaves and their palatability. Some in vitro studies (Cooke and Luxton, 1980; Cooke, 1983; Cortez and Hameed, 1988) suggest that earthworms prefer composted litter. The palatability of litters is usually estimated from the frequency and intensity of their burial in the soil. However, the disappearance of litter from the soil surface is not proof of its consumption by earthworms, as litter can be dragged into the soil by earthworms without being ingested or decomposed by microbial populations or be dragged into the soil by organisms other than earthworms.

Cortez and Hameed (1988) proposed an in vitro method based on the differentiation between earthworm and microbial activities using respiratory measurements in microcosms. Earthworms and micro-organisms simultaneously decompose both litter and the organic matter already present in the soil. These activities result in the release of CO_2 which can be measured. This technique, which enables the respiration by earthworms and micro-organisms on the litter and the organic matter in the soil to be distinguished and quantified, was used only on grass litters.

We studied the decomposition of leaf litters from four mediterranean tree species [sessile oak, *Quercus petraea* L. (SO); holm oak, *Q. ilex* L. (HO); sweet chestnut, *Castanea sativa* Mill (CH) and beech, *Fagus sylvatica* L. (B)] in the field at three sites, which differed in their earthworm biomass, for more than 2 y (Cortez, 1998; Cortez and Bouché, 1998; Cortez et al., 2000; Table 1). The litter disappearance rates presented two successive stages in the field: an initial relatively slow stage followed by a second faster stage. We assumed that the increase in the decay rate in the second stage did not depend on litter quality, but rather on earthworm activity and particularly on combined microbial and earthworm action. The low decay rate observed in the field during the first 345 d could be partly explained if the litters were initially unpalatable for earthworms and the litter decomposition was essentially due to micro-organisms. Then, during the following 419 d, the litters, which had become more palatable for earthworms after the preliminary microbial decomposition (Wright, 1972; Cooke

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Table 1
Mineralisation of the C litter and of the total C in the field (from Cortez, 1998). CM% = C mineralisation percentage; ±ew = with (+) or without (−) earthworms; CH = Sweet Chestnut; SO = Sessile Oak; HO = Holm Oak; B = Beech; f and c indicate fresh and composted litters. SE varied from 5.8 to 9.2%, $n = 10$

Species	Time of litter decay (-ew) (d)	Time of litter decay (+ew) (d)	Initial litter weight (g)	Final litter weight (g)	Initial litter C (%)	Final litter C (%)	Loss of C (g)	Litter CM% (%)	Litter CM% (% d^{-1})
CHf	345		4.00	2.12	48.4	38.9	1.1	57.5	0.17
CHc		419	2.12	0.46	39.4	33.0	0.7	81.8	0.20
SOf	345		4.00	2.16	47.6	41.8	1.0	52.6	0.15
SOc		419	2.16	0.62	38.5	35.5	0.6	73.6	0.18
HOf	345		4.00	2.84	48.7	44.5	0.7	35.1	0.10
HOc		419	2.84	1.23	40.4	39.9	0.7	57.2	0.14
Bf	345		4.00	3.48	47.0	40.7	0.5	24.7	0.07
Bc		419	3.48	2.56	41.5	38.0	0.5	32.6	0.08

Table 2
Initial C and N contents of the different litters; CH = Sweet Chestnut; SO = Sessile Oak; HO = Holm Oak; B = Beech; f and c indicate fresh and composted litters

Litter type	CH		SO		HO		B	
	f	c	F	c	f	c	f	c
C (%)	48.4	39.4	47.6	38.5	48.7	40.4	47.0	41.5
N (%)	1.03	1.16	0.97	1.08	0.77	0.95	0.68	0.81
C-to-N ratio	47.0	34.0	49.1	35.6	63.2	42.5	69.1	51.2

and Luxton, 1980; Cooke, 1983; Cortez and Hameed, 1988), were more rapidly decomposed. We considered that in this previous experiment 345 d on average were necessary to render the litters more palatable. These studies in the field have shown that the palatability of forest leaf litters for earthworms seems to increase with their residence time in the soil. To confirm this observation, we have applied the in vitro respiratory measurement technique to fresh and composted forest litters.

Our objectives were (1) to monitor the preference of earthworms for the same fresh and composted leaf litters using this respiration technique and (2) to relate these results to the decomposition rates of the same leaf litters in the field.

2. Materials and methods

2.1. Principle of the experiment

The principle of this experiment is based on CO_2 released by four kinds of microcosms: Soil (S), soil + earthworms (SE), soil + litter (SL) and soil + earthworms + litter (SEL). The different microcosms were used to develop a conceptual model of the action of micro-organisms and earthworms on litter and soil organic matter (Cortez et al., 1989).

We assume that $C–CO_2$ released by the SEL microcosm reflected the respiration of the (1) soil microflora, (2) earthworms and (3) litter decomposition by soil micro-organisms and earthworms. Using this experimental model it is, therefore, possible to calculate the respiration specific to each component of the system. If SEL is considered as the most complete system (100%), S represents the respiration of micro-organisms on soil organic matter ($M \rightarrow S$), ($SL - S$) the effect of soil microflora on litter ($M \rightarrow L$), $X = (SEL - SE) - (SL - S)$ the effect of earthworms on litter (analogous to litter palatability; $E \rightarrow L$) and ($SE - S$) the effect of earthworms on the soil organic matter ($E \rightarrow S$).

2.2. Soil samples

Soil (Cambisoil—FAO) was collected from the upper 5 cm of the A_1 horizon in an area situated in chestnut forest of the Cevennes hills (South of France) under humid mediterranean climatic conditions (clay 22%; silt 17.5%; sand 70%; C 1.96%; N 0.10%; pH H_2O 5.3; 380 m altitude). This soil came from the same experimental site (Anduze) used in an experiment where we studied the decomposition of leaf litter from the same four mediterranean tree species in the field (Cortez, 1998; Cortez and Bouché, 1998).

2.3. Earthworm collection

The experiments were carried out with the most representative earthworm species of this soil (*Nicodrilus meridionalis* L.) representing 73% of the total earthworm biomass (Cortez, 1998). Adult *N. meridionalis* were collected in the field by the formalin method (Raw, 1959), washed with H_2O, placed into the soil at 14°C and 98 Pa (pF 3) and fed for 3 or 4 weeks. By then, the earthworms were fully active. They were hand-picked and adult earthworms of similar weight (difference between 4 and 7%) were used for the experiment.

2.4. Litter samples

We studied the decomposition of (1) fresh litters from sweet chestnut (CHf), sessile oak (SOf), holm oak (HO_f), and beech (Bf) and (2) composted litters (CHc, SOc, HOc, Bc) composed of the same litter types allowed to decompose for 2 months in vitro under optimal humidity and temperature conditions.

The composted litters were prepared as follows: about 350 g of leaves from each fresh litter cut in pieces (1–2 cm^2), were incubated by rewetting the material until saturation with a mixture of mire water (obtained from the site soil by shaking with water; w/v = 2%) and left to decompose for 60 d at 28°C. The water content was controlled by weighing every 4–5 d and readjusted with the mire water if necessary. After washing the four composted litters were dried at 60°C in an oven for 48 h. Total organic C was determined by dry combustion (Carmograph 12M) and total N by the Kjeldahl method. The initial C and N contents of the litters are shown in Table 2.

2.5. Experimental procedure

The air-dry soil was sieved (2 mm), homogenized and moistened to 98 Pa. Equal amounts of the moist soil (700 g air-dry equivalent) were put into 108 hermetically

sealed 1.5-l vessels. The following treatments (summarised in Table 3) were used:

1. 24 replicates with soil, one earthworm and fresh litter (6 × CHf; 6 × SOf; 6 × HOf; 6 × Bf) cut in small pieces (1–2 cm^2). To avoid fungal growth the litter was added as and when required by earthworms (SEL).
2. 24 replicates with soil, one earthworm and composted litter (6 × CHc; 6 × SOc; 6 × HOc; 6 × Bc) cut in small pieces (1–2 cm^2). The composted litter was added as and when required by earthworms (SEL).
3. 24 replicates with soil and 2 g of fresh litter cut in small pieces (1–2 cm^2) (6 × CHf; 6 × SOf; 6 × HOf and 6 × Bf) (SL).
4. 24 replicates with soil and 2 g of composted litter cut in small pieces (1–2 cm^2) (6 × CHc; 6 × SOc; 6 × HOc; 6 × Bc) (SL).
5. six replicates with soil and one earthworm (SE).
6. six replicates with soil only (S).

Each vessel contained a flask with 20 ml 0.5 M NaOH to absorb CO_2. Respirometry of the CO_2 absorbed in the alkaki was measured by flow colorimetry (Chaussod et al., 1986). The flasks were changed every 2 or 3 d. The vessels were kept in darkness for 31 d at 14°C, which is the best temperature for earthworm activity (more feeding, faster growth but the reproduction was not measured). These experimental variables were determined in a previous experiment (Cortez et al., 1989). Moisture was maintained throughout the experiment by watering as required. After 31 d, all earthworms were collected from the microcosms, washed with distilled water, dried with filter paper and weighed (g live worm weight).

2.6. Field methodology

Full details of this field experiment are presented in Cortez (1998). In short: leaf litter of holm oak (*Q. ilex* L.), sessile oak (*Q. petraea* L.), sweet chestnut (*C. sativa* Mill.) and beech (*F. sylvatica* L.) was collected in the field (Cevennes hills, South of France). Samples of the air-dried litters (4 g) were cut into small pieces (1–3 cm^2 each) and placed in polyester net litterbags (16 × 12 cm; 0.5 cm mesh) and left to decompose for more than 2 y (764 d) on the soil surface of three sites differing by their earthworm biomass. For each kind of litter 120 litterbags were arranged in 10 small quadrat squares (each containing 12 litterbags) at each experimental site. One litterbag of each litter was sampled from each quadrat every 2 months. The recovered materials were (1) air-dried for 2 or 3 weeks and then dried at 40°C to constant weight and (2) carefully separated by hand from soil particles and weighed.

2.7. Statistical analysis

Means and SE, linear regressions were carried out using Excel software (Microsoft, Version 7.0).

3. Results

3.1. CO_2–C release from microcosms

Our experimental results, based on CO_2 released by the different microcosms allow development of a conceptual model of the action of micro-organisms and earthworms on litter and soil organic matter. So Fig. 1 shows the relative proportions (%) of C–CO_2 released from the different microcosms and demonstrates the effects of litter type, composting and *N. meridionalis*.

Table 4 shows that for a given plant species, the quantities of C–CO_2 released after 31 d of incubation were always significantly higher for composted than for the fresh litters. The increases in C–CO_2 release between composted litters and fresh litters were as follows: CH = +29.1%; SO = +24.5%; HO = +21.2%; B = +10.8%.

The difference in the CO_2 respired between the SL and S microcosms expresses the action of soil micro-organisms on the different litters (M → L). The quantity of litter decomposed by microbial action alone was also always higher for composted litters (CH = +29.7%; SO = +49.7%; HO = +56.8%; B = +23.0%). The amounts of C–CO_2 released by the complete system (SEL) and by M → L were classified in the following order: CH > SO > HO > B for both types of litter. The percentage C mineralisation attributable to micro-organisms and earthworms can be calculated from these results. Table 5 shows that the proportion of C in the litters mineralised by the microflora (M → L) was higher for the composted litters (from 1.1 to 1.4 times greater than for fresh litters).

The E → L compartment is of great importance since it gives an idea of the relative palatability of the different litters for the earthworms. For example, the CHc and SOc litters seemed to be the most palatable since their E → L compartments were 21.4 and 15.4 mg C–CO_2 100 g^{-1} ds g^{-1} live worm, respectively, compared to 9.1 and 7.6 mg C–CO_2 100 g^{-1} ds g^{-1} live worm for CHf and So_f (Table 4). The same phenomenon was found when HOc and Bc were compared with HOf and Bf. The quantity of C–CO_2 released by E → L showed that earthworms accounted for 15.2–17% of the decomposition of fresh litters with SOf and CHf but their effects were negligible with HOf and Bf. In contrast, composted litters were more consumed by earthworms and, therefore, seemed to be more palatable (24.7–31% of total respiration for SOc and CHc; 6–8.8% for Bc and HOc). Table 5 shows that the proportion of C in the litters mineralised by the earthworms (E → L) was much more higher for the composted litters (from 2 to 25 times greater than for fresh litters).

3.2. Litter consumption

It is noteworthy that, after 31 d, all the litters had disappeared from the soil surface of all microcosms. The daily consumption of litters varied with litter type (Table 6). It

Table 3
Experimental procedure. SEL = soil + earthworm + litter; SL = soil + litter; SE = soil + earthworm; S = soil only. ew = earthworm; CH = Sweet Chestnut; SO = Sessile Oak; HO = Holm Oak; B = Beech; f and c indicate fresh and composted litters

Microcosm type	SEL (f)				SL (f)				SE	S	SEL (c)				SL (c)			
Number of microcosms	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Soil weight (g)	700	700	700	700	700	700	700	700	700	700	700	700	700	700	700	700	700	700
Number of ew	1	1	1	1	0	0	0	0	1	0	1	1	1	1	0	0	0	0
Litter type	CHf	SOf	HOf	Bf	CHf	SOf	HOf	Bf	0	0	CHc	SOc	HOc	Bc	CHc	SOc	HOc	Bc
Litter quantity	Added when required by ew				2g	2g	2g	2g	–	–	Added when required by ew				2g	2g	2g	2g

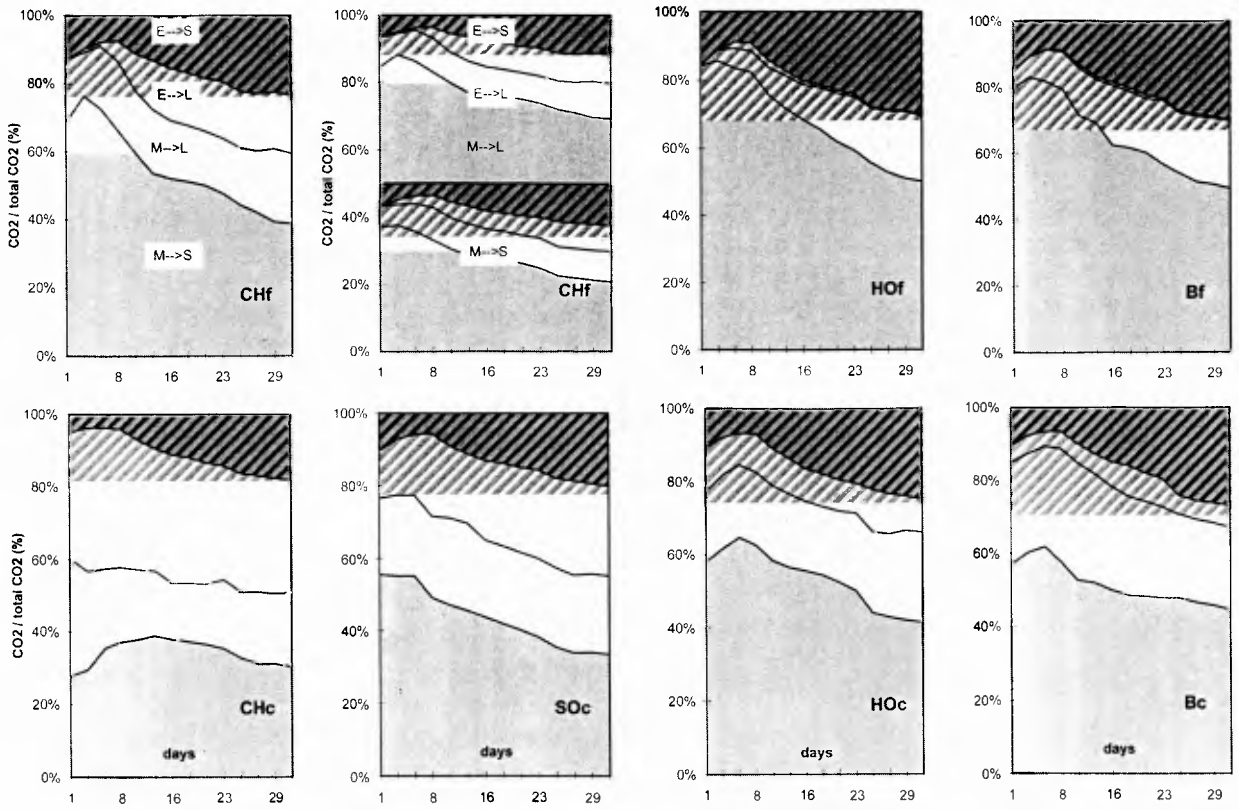

Fig. 1. Relative proportions (%) of carbon dioxide released from microcosms containing either forest topsoil alone (M → S), topsoil with fresh or composted leaf litter of one of four different species of forest tree (M → L), topsoil with the eartworm (*Nicodrilus meridionalis*) (E → S) or topsoil with fresh or composted leaf litter of one of the four different species of forest tree and *N. meridionalis* (E → L) (see Table 4). S = Forest topsoil; L = Leaf litter; M = micro-organisms; E = *N. meridionalis*; f = fresh leaf litter; c = composted leaf litter. CH = Sweet Chestnut, HO = Holm oak; SO = Sessile oak; B = Beech.

was lower with the fresh litters than with the corresponding composted litters. However the consumption rates varied from 10.7 to 21.4 mg g^{-1} live worm d^{-1}. They were comparable to those reported by Shipitalo et al. (1988) for *Lumbricus terrestris* and *L. rubellus* (from 5 to 52 mg g^{-1} live worm d^{-1} at 15°C depending upon the litter quality and the earthworm species). 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 d^{-1} at 21°C for *L. terrestris*. These results demonstrated the effect of temperature on litter consumption.

3.3. Earthworm biomass changes

The changes in earthworm biomass over 31 d of incubation in relation to the quantity and quality of the litter provided are shown in Table 6. The biomass increased strongly with the composted litters (from 3.5% for Bc to 14.9% for CHc) and with certain fresh litters such as CHf (+2.8%) and SOf (+1.6%), but there was a large decrease in earthworm biomass with HOf (−14.2%) and Bf (−13.2%). This weight loss was greater than that recorded in the SE microcosms (−8.4%), confirming the lack of palatability of these litters or/and the occurrence of toxic or inhibitory substances in the HOf and Bf litters. Furthermore the gain in earthworm biomass was closely correlated (1) with litter palatability (Table 7, nos. 1 and 2) and (2) with the N consumed by earthworms (ECLN; Table 7, nos. 5 and 6), and the N incorporated into their tissues (EILN; Table 7, nos. 7 and 8).

4. Discussion

4.1. In vitro experiments

Our results agree with those of Satchell and Lowe (1967) who showed that there was a close correlation between the preferred food and its chemical quality, for example its contents of N, phenolic compounds and carbohydrates. The highest weight losses were found with fresh litters HOf and Bf (Table 6) which seem to be the least palatable. It is known that HOf leaves contain aromatic phenolic compounds and primary and carboxylic alcohols that are potentially toxic for earthworms (Brattsen, 1979). Edwards and Heath (1975) reported that leaves containing high concentrations of water-soluble polyphenols are the least consumed. The decrease in biomass resulting from the consumption of HOf and of Bf was greater than that resulting from the consumption of soil on its own (Table 6). It, therefore, seems that the relative toxicity of HOf and Bf litters was one of the possible causes of their poor assimilation by *N.*

Table 4
Cumulative values of total $C–CO_2$ (in mg $C–CO_2$ 100 g^{-1} dry soil g^{-1} live worm) released by the effect of micro-organisms on soil (M → S), micro-organisms on litters (M → L), earthworms on litters (E → L) and earthworms on soil (E → S). CH = Sweet Chestnut; SO = Sessile Oak; HO = Holm Oak; B = Beech; f and c indicate fresh and composted litters

Time (d)	M → S	M → L	E → L	E → S	Sum
CHf					
1	2.4	0.6	0.0	0.4	3.4
6	8.0	2.0	0.1	0.9	11.0
10	10.5	3.3	1.9	2.0	17.7
20	16.0	5.1	5.0	6.0	32.0
31	20.8	11.0	9.1	12.6	53.5
CHc					
1	2.4	2.8	3.0	0.4	8.5
6	8.0	5.0	8.7	0.9	22.5
10	10.5	5.4	9.9	2.0	27.8
20	16.0	7.3	14.7	6.0	44.0
31	20.8	14.3	21.4	12.6	69.0
SOf					
1	2.4	0.4	0.0	0.4	3.2
6	8.0	1.9	0.5	0.9	11.2
10	10.5	3.2	1.6	2.0	17.4
20	16.0	5.1	3.6	6.0	30.7
31	20.8	9.0	7.6	12.6	50.0
SOc					
1	2.4	0.9	0.6	0.4	4.2
6	8.0	3.2	2.4	0.9	14.4
10	10.5	5.4	4.5	2.0	22.4
20	16.0	8.5	9.4	6.0	39.9
31	20.8	13.5	15.4	12.6	62.3
HOf					
1	2.4	0.0	0.0	0.4	2.8
6	8.0	0.5	0.2	0.9	9.5
10	10.5	1.1	0.3	2.0	14.0
20	16.0	3.7	0.2	6.0	25.9
31	20.8	8.0	0.2	12.6	41.6
HOc					
1	2.4	0.8	0.5	0.4	4.1
6	8.0	2.4	1.1	0.9	12.3
10	10.5	3.7	1.8	2.0	18.1
20	16.0	6.0	2.6	6.0	30.6
31	20.8	12.5	4.4	12.6	50.4
Bf					
1	2.4	0.1	0.0	0.4	3.0
6	8.0	0.9	0.0	0.9	9.8
10	10.5	2.1	0.1	2.0	14.8
20	16.0	4.4	0.2	6.0	26.6
31	20.8	8.5	0.2	12.6	42.1
Bc					
1	2.4	1.2	0.2	0.4	4.1
6	8.0	3.5	0.5	0.9	12.9
10	10.5	6.5	1.0	2.0	20.1
20	16.0	8.6	2.6	6.0	33.2
31	20.8	10.5	2.8	12.6	46.7

meridionalis. The highest biomass gains were obtained with composted litters, particularly with CHc (+14.9%) and SOc (+10.1%). This weight increase was possibly related to the C-to-N ratio of the litters which was lower in composted litters (see Table 2), i.e. to quality of the leaf composted vs fresh. However there is also a possibility that there was a direct relation between the gain in biomass and the N availability. For example, despite its lower N content (0.81%), Bc was better used than CHf which had a lower C/N ratio (47) and a higher but maybe less assimilable N content (1.03%). This concept of assimilability was demonstrated by Abbott and Parker (1981). They showed that a N-poor diet led to a decrease in earthworm biomass which could not be compensated by a supply of inorganic N from the soil, but could be overcome by providing a protein-rich food, that is more readily used by earthworms (Lee, 1985). The high N throughput of these animals (Férrière and Bouché, 1985; Hameed et al., 1994) makes them dependent on resources riches in assimilable N.

4.2. Carbon mineralisation percentage of litters

We have seen that micro-organisms mineralised more of the composted than the fresh litters but in a lower percentage than by earthworms (Table 4). However there was a close correlation between the respective effects of micro-organisms and earthworms on the two types of litter (Table 7, nos. 11 and 12). This is entirely normal, since the action of an earthworm on a substrate is in fact a combined earthworm–microbial action, as earthworms produce the conditions needed for intense microbial growth. So these results show that composted litter to be more readily mineralised by micro-organisms, and even more so by earthworms, than fresh litter. These ratios were greater, the more difficult the fresh litter was to break down. The greater the C mineralisation rate of the litter, the more it became palatable for earthworms (Table 7, nos. 9 and 10). This relationship was stronger with composted litters. So earthworms consumed almost no fresh litters of HO and B, but following composting they were consumed. During the composting, it is likely that some of the compounds that are toxic for animals are eliminated from the litters and that the litters are also invaded by microflora, especially fungi. This is in agreement with the work of Wright (1972) who showed that the presence of micro-organisms enhanced feeding rates of earthworms.

4.3. Carbon and nitrogen balance in relation to the types of litter provided

The equation for the energy flow passing through a living organism can be expressed as (Lee, 1985): C (consumption) = P (production) + R (respiration) + E (elimination).

This equation is usually used to calculate the energy balance (Bolton and Phillipson, 1976a,b; Lavelle, 1977). We thought that it could be used to establish a balance in relation to (1) the C and N consumed and excreted and (2) the C respired, by assuming that the C and N originating from the soil were only slightly metabolised by earthworms. To do this, we considered that compartment E included all the earthworm 'outputs', namely surface cast and underground faeces and also losses by cutaneous secretions (mucus, NH_3, etc.) that are rich in C and N (Cortez and Bouché, 1987). To give an example of such a calculation,

Table 5
Mineralisation of the C litter and of the total C in vitro (%). M → L = effect of micro-organisms on litter and E → L = effect of earthworm on litter (see Table 3); CM% = C mineralisation percentage; (*) expressed in mg CO_2–C 100 g^{-1} dry soil g^{-1} live worm ;(#) = [(initial live worm biomass + final live worm biomass)/2 − SE varied from 4.5 to 8.9%; $n = 6$]; CH = Sweet Chestnut; SO = Sessile Oak; HO = Holm Oak; B = Beech; f and c indicate fresh and composted litters

Plant species	Added litter (mean) (g)	Litter C (%)	Litter C (mg)	Earthworm biomass (g) (mean ± SE) (#)	Added litter C (*)	C loss (1) M → L (*)	M → L CM%	C loss (2) E → L (*)	E → L CM%	C total loss (1 + 2)	Total litter CM% (%)	Litter CM% (% d^{-1})
CHf	3.0	48.4	1452	5.95 ± 0.26	34.9	11.0	31.5	9.1	26.1	20.1	57.6	0.65
CHc	4.0	39.4	1576	6.04 ± 0.35	37.3	14.3	38.3	21.4	57.4	35.7	95.7	1.15
SOf	2.6	47.7	1240	5.33 ± 0.31	33.2	9.0	27.2	7.6	22.9	16.6	50.1	0.54
SOc	4.0	38.5	1540	6.09 ± 0.38	36.1	13.5	37.4	15.4	42.6	28.9	80.0	0.93
HOf	1.7	48.7	828	5.20 ± 0.28	22.7	8.0	35.1	0.2	0.7	8.2	35.9	0.26
HOc	2.7	40.4	1091	6.00 ± 0.35	26.0	12.5	48.1	4.5	17.3	17.0	65.5	0.55
Bf	1.9	47.0	893	5.15 ± 0.32	24.8	8.5	34.4	0.2	0.8	8.7	35.3	0.28
Bc	2.7	41.5	1121	5.88 ± 0.36	27.2	10.5	38.6	2.8	10.3	13.3	48.9	0.43

Table 6
Quantity of litter added, daily plant litter consumption and change in earthworm biomass (g live worm) after 31 d incubation with the different litters. Daily litter consumption expressed in mg g^{-1} live worm d^{-1}; m ± SE = mean ± SE; CH = Sweet Chestnut; SO = Sessile Oak; HO = Holm Oak; B = Beech; f and c indicate fresh and composted litters

	Litter type								
	CH		SO		HO		B		Soil
	f	c	f	c	f	c	f	c	
Total plant litter added (g) m ± SE	3.0 ± 0	4.0 ± 0	2.6 ± 0.2	4.0 ± 0	1.7 ± 0.2	2.7 ± 0.2	1.9 ± 0.5	2.7 ± 0.2	
Daily litter consumption m ± SE	16.3 ± 0	21.4 ± 0	15.8 ± 1.2	21.2 ± 0	10.7 ± 1.2	14.0 ± 1.3	11.9 ± 2.9	14.8 ± 1.3	
Change in earthworm biomass m(%) ± SE	(+)2.8 ± 10.3	(+)14.9 ± 11.3	(+)1.6 ± 8.2	(+)10.1 ± 12.8	(−)14.2 ± 22.9	(+)3.8 ± 31.3	(−)13.2 ± 18.0	(+)3.5 ± 31.5	(−)8.4 ± 14.1

Table 7
Linear regressions between the different parameters of the experimental systems. r^2 = coefficient of determination; EBMV = earthworm biomass variation; M → L = effect of soil microflora on litters; E → L = litter palatability; CLN = litter N (mg g^{-1}) consumed by earthworms and microflora; ECLN = litter N consumed by earthworms (mg g^{-1} live worm); EILN = litter N incorporated in earthworm biomass (mg g^{-1} live worm); CM% = the C mineralisation percentage; f and c indicate fresh and composted litters. Sig = significance; ***$P < 0{,}001$; **$P < 0{,}01$; $n = 24$

Regression no.		x	y	Equation	r^2	Sig
1	f	EBMV (%)	E → L (%)	$y = 0.954x + 13.763$	0.958	***
2	c	EBMV (%)	E → L (%)	$y = 2.036x + 1.094$	0.904	***
3	f	EBMV (%)	CLN	$y = 0.149x + 4.585$	0.943	***
4	c	EBMV (%)	CLN	$y = 0.327x + 3.039$	0.864	***
5	f	EBMV (%)	ECLN	$y = 0.143x + 4.347$	0.936	***
6	c	EBMV (%)	ECLN	$y = 0.293x + 3.024$	0.842	***
7	f	EBMV (%)	EILN	$y = 0.005x + 0.239$	0.832	***
8	c	EBMV (%)	EILN	$y = 0.034x + 0.014$	0.912	***
9	f	CM%	E → L (%)	$y = 0.793x - 41.149$	0.881	***
10	c	CM%	E → L (%)	$y = 0.262x - 10.887$	0.832	***
11	f	M → L (%)	E → L (%)	$y = 2.350x - 17.098$	0.549	**
12	c	M → L (%)	E → L (%)	$y = 3.761x - 37.051$	0.612	***

take the case of S + E + CHf. The weight of litter consumed by the earthworm per microcosm is equal to the total weight of litter ingested (3.0 g, Table 5) minus the weight of litter decomposed by soil micro-organisms ($11.0 \times 7 \times 100/48.4 = 158$ mg, see Table 5 for specific data), i.e. $3000 - 158 = 2842$ mg. Knowing that CHf litter contains 48.4% C and that the mean weight of an earthworm is 5.95 g, the quantity of litter C on which the earthworm exerts its action is: $2842 \times 0.484/5.95 \times 31 = 7.45$ mg C g^{-1} live worm d^{-1} which gives a $C–CO_2$ release of 2.05 mg C g^{-1} live worm d^{-1}. As the increase in the weight of the earthworm was 0.09 mg C g^{-1} live worm d^{-1}, the C exiting the system is deduced by subtraction, i.e. $7.45 - (2.05 + 0.09) = 5.31$ mg C g^{-1} live worm d^{-1}.

In this experiment with *N. meridionalis*, these results reveal the following points (Table 8).

1. The assimilation rates of C and N varied depending on the nature of the litters. They were always higher with composted litters compared to fresh litters and were zero for HOf and Bf. There was also a highly significant correlation between changes in earthworm biomass and the quantities of N that they incorporated (Table 7, nos. 7 and 8).
2. The quantity of C respired compared to the quantity of C incorporated by the earthworm (Table 8, column 8), showed that energy expenditure required for incorporating fresh litters was higher. This observation was confirmed (Table 8, columns 9 and 15) by the fact that the proportions of C and of N incorporated by earthworms was always higher when these elements came from composted litters, which implies that composted litters were much more palatable and better assimilated than fresh litters. Cooke and Luxton (1980) suggested that "microbial contamination of dead plant material released phagostimulants attractive to earthworms". In our experiment composted litters were probably more colonised by micro-organisms than fresh litters and this hypothesis could be an explanation for our results.
3. We did not measure cast production, but it is possible to estimate the C and N output using the equation of Lee (1985). As fresh casts contain about 5% C, we calculated the cast production rates (Table 8, column 7) which varied from 34 to 130 mg g^{-1} live worm d^{-1} for *N. meridionalis* and were similar to those reported by Shipitalo et al. (1988) for *L. terrestris*. Our experiment shows that *N. meridionalis* lost weight when feeding on unpalatable litters but produced more casts. Cast production depends on climatic conditions and on earthworm diet. If they do not find the nutrients required for their metabolism, earthworms tend to explore more soil (Abbott and Parker, 1981; Lee, 1985; Cortez et al., 1989) and, therefore, produce more casts. Differences in earthworm diet also resulted in a modification in cast composition, the C-to-N ratio of casts varying with diet from 11.7 to 53. It was generally lower with the most palatable litters and higher with the others (Table 8, column 17). These results agree with those reported by Cortez et al. (1989) who studied the C and N transfer in soil with *L. terrestris* fed with wheat straw.
4. It is noteworthy that the C-to-N ratios of compounds assimilated by earthworms varied between 5 (SOc) and 5.4 (Bc). These C-to-N ratios are similar to those of peptides and aminoacids that could indicate an absorption of many organic compounds in the form of peptides or amino acids.

4.4. Comparison of laboratory data with results of field experiments

The C mineralisation percentages of the litters in vitro and in the field (Tables 1 and 5) were similar, evolved in the same way and were always higher with the composted than with the same fresh litters. In the field we assumed that the effect of earthworms was negligible before 345 d

Table 8
C and N balance in the different compartments of the model in vitro experiments. Columns 3, 4 in mg C g^{-1} live worm d^{-1}; columns 5, 6 in mg C g^{-1} live worm d^{-1}; column 7 in mg g^{-1} live worm d^{-1}; columns 13, 14 in mg N g^{-1} live worm d^{-1}; ew = earthworms; BM = biomass; CH = Sweet Chestnut; SO = Sessile Oak; HO = Holm Oak; B = Beech; f and c indicate fresh and composted litters

1 Litter type	2 Litter C (%)	3 Ingested C by ew	4 $C–CO_2$ release E → L	5 C incorporated in ew BM	6 Calculated C output (casts)	7 Cast calculated production	8 (4)/(5)	9 (5)/(3) (%)	10 Litter N (%)	11 Ingested N by ew	12 Ew N mean (%)	13 N incorporated in ew BM	14 Calculated N output (casts)	15 (13)/(10) (%)	16 C-to-N ratio of incorporated material	17 C-to-N ratio of casts
CHf	48.4	7.45	2.05	0.09	5.31	106	22.8	1.2	1.03	0.16	1.8	0.02	0.14	12.5	4.5	37.9
CHc	39.4	7.80	5.68	0.43	1.69	34	13.2	5.5	1.16	0.23	1.9	0.09	0.14	39.1	4.8	12.1
SOf	47.7	7.12	1.72	0.05	5.35	107	34.4	0.7	0.97	0.14	1.9	0.01	0.13	7.1	5.0	41.2
SOc	38.5	7.80	4.08	0.29	3.43	69	14.1	3.7	1.08	0.22	1.9	0.06	0.16	27.3	4.8	21.4
HOf	47.0	5.21	0.05	−0.49	5.65	113	–	–	0.77	0.08	2.0	−0.10	0.18	–	–	29.1
HOc	40.4	5.64	1.18	0.11	4.35	87	10.7	2.0	0.95	0.13	1.8	0.02	0.11	15.4	5.5	39.5
Bf	47.0	5.21	0.05	−0.45	5.61	112	–	–	0.68	0.08	2.0	−0.09	0.17	–	–	33.0
Bc	41.5	5.95	0.74	0.11	5.10	102	6.7	1.8	0.81	0.12	1.8	0.02	0.10	16.7	5.5	51.0

Table 9
Linear regressions between the carbon mineralisation percentage (CM%) in vitro and in the field. M → L = effect of soil microflora on litter; E → L = litter palatability; r^2 = coefficient of determination; Sig = significance; ***$P < 0.001$

	x	y	Equation	r^2	n	Sig
CM% of fresh litter	M → L (%)	E → L (%)	$y = 1.310x - 16.097$	0.895	24	***
CM% of composted litter	M → L (%)	E → L (%)	$y = 1.049x - 15.047$	0.942	24	***

because of the litter unpalatability; the similarity of the C mineralisation percentages in vitro and in the field confirm this assumption. Highly significant linear regressions have been fitted between C mineralisation percentages in vitro and in the field (Table 9). So the in vitro model could provide some insight into the behaviour of micro-organisms and of the micro-organism-earthworm association on different kinds of litter and on soil organic matter.

4.5. Conclusion

Our contribution to the understanding of the relationship between litter palatability for earthworms and its degree of decomposition can be summed up as follows.

The proposed respirometric method provides an estimate of the biological activity of earthworms and soil micro-organisms when decomposing different litter species and litter palatability for earthworms can be easily evaluated. Estimates can be made of the relative contributions of each of the system's compartments (Fig. 1).

However the microcosms used present some disadvantages. The $C-CO_2$ released by the SL microcosm (measurement of the effect of micro-organisms on litter) represents the respiration of the topsoil only. But earthworms also bury the litter in soil and this method underestimates the effect of micro-organisms on litter at depth. Despite this disadvantage, it can be used to test the palatability of many litters.

For the same litter species, the composted litters and the litters undergoing a preliminary microbial decomposition in the soil became more palatable for earthworms since they always resulted in an increase of earthworm biomass.

The decomposition rates of litters were highly correlated in controlled conditions and in the field. Hence these models could provide a quick estimate of the decomposibility and palatability for earthworms of different kinds of litters.

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