



EXEMPLAIRE RÉSERVÉ

FIELD DECOMPOSITION OF LEAF LITTERS: EARTHWORM–MICROORGANISM INTERACTIONS —THE PLOUGHING-IN EFFECT

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Summary—We describe a new decomposition model including the consequences of earthworm mechanical activity, especially the ploughing-in effect. In an experiment we tested this ploughing-in effect on the disappearance of leaf litter from four tree species (sessile oak, *Quercus petraea* L., holm oak, *Quercus ilex* L., sweet chestnut, *Castanea sativa* Mill and beech, *Fagus sylvatica* L.) during two 2 y exposure at the site of Anduze which contained the highest earthworm biomass. Our experiment, carried out with leaf litter placed in litterbags allowing (0.5 cm mesh size) or preventing (0.1 cm mesh size) the access of earthworms, confirms (1) the rates of litter decomposition observed in the first experiment and (2) the important effect of earthworms on litter decomposition (from 20.5% to 30.2% depending on the litter). The breakdown of the different litters by soil fauna varied from 24.2% to 40.6% depending on the litters after 2 y exposure in the field at Anduze and the litter decomposition rate was increased by a mean factor of about 5.2. We describe a technique for detecting litter contamination attributed to earthworm casts. This technique is based on the change of the litter total weight loss (TWL)-to-litter total C loss (TCL) ratios which varied from 0.652 to 0.915 in the presence of earthworms and were close to 1 (from 0.952 to 0.995) in the absence of earthworms. We assumed that this large change in these ratios indicated a mineral contamination of litters due to earthworm casting. This hypothesis was confirmed by the litter ash contents varying from 2.6% to 9.6% with earthworms and from 1.3% to 2.1% without earthworms only. These results point out the ecological importance of contamination of the litter by casts during decomposition. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The aim of our study was to compare the rate of disappearance of four different forest litters (sessile oak, *Quercus petraea* L.; holm oak, *Quercus ilex* L.; sweet chestnut, *Castanea sativa* Mill and beech, *Fagus sylvatica* L.) at three forest sites. To do this the litterbag method was adopted, using bags with a large mesh that allowed activity both by macro- and micro-organisms (Cortez, 1998). The analysis of the results of this first experiment showed that, when there was intense anecic earthworm activity, the litter disappeared at two successive rates and not at a single rate. This earthworm activity profoundly changed the surrounding conditions to permit maximal microbial decomposition (Cortez, 1998) and litter disappearance, phenomena that cannot be observed in microcosms. In microcosms earthworm and microorganism activities depend first on the experimenter's choice i.e. generally with (1) no diel rhythm (no variations in light, temperature and moisture), (2) no seasonal rhythms affect-

ing earthworm activity by an endocrinal regulation of the diapause. In microcosms, earthworms modify their feeding behaviour and their physical activity to survive under these abnormal conditions (Bouché and Al-Addan, 1997). Moreover, microcosm results are difficult to falsify in true field systems.

The standard conceptual model which simply combines microbial decomposition with the process of ingestion and digestion by earthworms, therefore no longer suffices in this case. Account has to be taken of the mechanical role of transport, comminution and mixing by earthworms over the entire soil profile or over that of the production of crumbs from casts. In order to obtain an understanding of decomposition processes from the scattered and very rare field observations—the only ones consistent with mechanical earthworm behaviour—we propose substituting the classical scheme described above, with a new integrated decomposition scheme.

The general decomposition scheme (Fig. 1) distinguishes two main mechanisms: (1) the microbiota decomposition process and (2) a lumbrico-microbiota process where earthworms are active.

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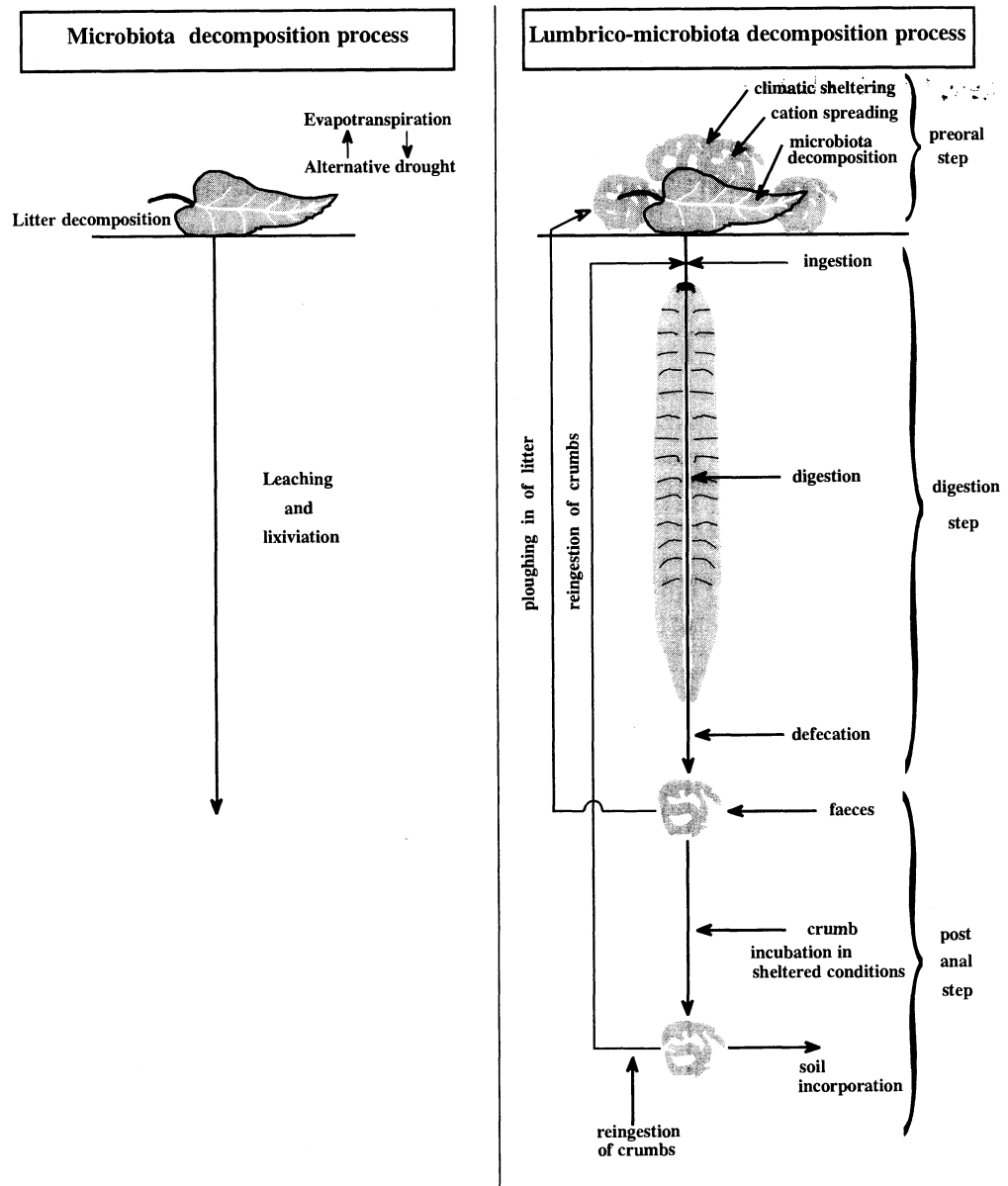


Fig. 1. Conceptual model of litter decomposition by earthworms.

The microbiota decomposition process

Without topsoil litter feeders (mostly anecic earthworms) decomposition depends on climatic conditions at each experimental site. The influx of new litter modifies this microclimate by ploughing-in the previous layer. Decomposition depends entirely on microbial activity and possibly on meso-fauna activity (Acari, Collembola, Enchytraeidae, epigeous earthworms). There is no animal mixing with mineral layers but only lixiviation and leaching. Decay rates are mainly dependent on litter age, composition and seasonal pedoclimatic variations.

The lumbrico-microbial process

The pre-oral stage In the presence of anecic and epi-anecic earthworms the microclimatic conditions of microbial activity are changed by three mechanisms:

1. The surface litter accumulation mechanism: the pulling of organic debris towards the burrow mouth. Accumulated undegraded debris results in a special microhabitat around these openings (Lamparsky, 1985).
2. The litter burrow lining mechanism: the capacity of some earthworm species, particularly the epi-

anecics (*Lumbricus terrestris*) to pull non-ingested organic matter into their burrows creates a specific sheltered microbial microhabitat in the soil (Hensen, 1877).

3. The litter ploughing-in by cast mechanism: the deposit of surface casts on and into the litters ploughs it in. This mechanism of burying was thoroughly described by Darwin (1837). This litter ploughing-in modifies pedoclimatic conditions in the pre-oral microbial field incubation stage and to some extent the results of the post-anal field incubation, as seen below.

The two first mechanisms and particularly the second are subject to litter palatability unlike the third mechanism.

The earthworm digestion stage Digestion can be divided into three mechanisms: (1) ingestion, (2) digestion itself with pedo-intestinal grinding and mixing and (3) cast production.

1. Ingestion and gripping by the mouth depend on the palatability of organic food (litter) subjected to the first microbial pre-oral stage (Cortez *et al.*, 1989; Cortez *et al.*, 1993) and on organo-mineral food of the post-anal stage described below. Mechanical resistance to ingestion is another limiting factor linked to debris and earthworm sizes. Large earthworms can ingest very tough leaves while smaller earthworm species prefer leaves softened by microbial decomposition (Wright, 1972).
2. The digestion itself provides a faster decomposition involving a huge C loss *in situ* (Bouché *et al.*, 1983). Furthermore earthworms assimilate a high quantity of N that they excrete essentially by skin secretions (Ferrière and Bouché, 1985; Cortez and Bouché, 1987; Hameed *et al.*, 1994). In the gut there is a change in pH, an influx of water and excretion of mucus which could play a priming effect role (Martin *et al.*, 1987). Organic matter is crushed during digestion.
3. Casting. Anecic earthworms, by mixing organic matter with minerals and soil microorganisms, blend a mixture that is finally cast into the soil (60 to 90% of faeces) but also onto the topsoil. In contrast epigeous earthworms excrete their organic casts on the soil surface only.

The post-anal stage The post-anal decomposition of faeces resulting in stable crumbs is characteristic of mull soils and is mediated by earthworms according to two mechanisms: microbial modification of faeces to stable crumbs and re-ingestion by earthworms.

1. Post-anal decomposition, i.e. the soil crumb capacity, depends on the nature of the initial litter and of the gut mixing, producing a blend on which a post-anal microbial activity flourishes

(Parle, 1963). This process depends also on the amount of cast production and season, i.e. of microsite conditions affect crumb stability (Al-Addan, 1992).

2. The re-ingestion of crumbs was demonstrated by a re-interpretation of data from Dietz and Bottner (1981) who studied the ingestion and assimilation of ^{14}C labelled litter by the earthworm *Nicodrilus meridionalis*. This re-interpretation demonstrated the re-ingestion of the ^{14}C labelled crumbs that had been deposited in soil (Bouché *et al.*, 1987). Independently, Bouché *et al.* (1983) estimated *in situ* the ingested material of *Nicodrilus velox* as: 19.9% of litter and 53.9% of re-ingested former faeces (crumbs) accounting for 61.4% and 34.6% of the ingested C respectively.

This general model, with two processes, three steps and eight mechanisms, has been well described for the microbiota decomposition process and the earthworm digestion stage. The post-anal stage is poorly documented while the pre-oral stage has been described with no quantification. Our aim was to study the effect of the ploughing-in mechanism on changes and disappearance of litter. We used the results of the first experiment (Cortez, 1998), in which the processes were mixed, with the results of the second experiment in which microbiota and lumbrico-microbiota processes were studied separately by the use of different mesh-size litterbags.

MATERIALS AND METHODS

This experiment was carried out at the Anduze site only, because this site contains the higher earthworm biomass. The situation of the site and the soil characteristics are given in Cortez (1998) (Table 1).

Litter samples

The same material (litter species) and arrangement in the field were carried out as in the first experiment. However litters were placed simultaneously two kinds of different mesh size litterbags, to separate the microbiota (wire net litterbags; 16 × 12 cm; 0.1 cm mesh) and the lumbrico-microbial (polyester net litterbags; 16 × 12 cm; 0.5 cm mesh) decomposition processes. The litterbags which were left on the soil surface for 760 d, at Anduze, because this site contains the highest earthworm biomass. 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 and (3) finally ground for C and N analysis and ash content determination.

Earthworm collection

See Cortez (1998) (Table 2).

Analytical methods

Litter total N was determined by the Kjeldahl method and colorimetry flow analysis and total litter organic C by dry combustion (Carmograph 12M). Ash content percentages were determined after a first drying of litter samples at 70°C for 24 h and combustion of organic matter in an oven at 750°C for 24 h.

Statistical analysis

Fitting to linear and parabolic and logistic functions, means and SE were carried out using GRAPHPAD software (GraphPad InPlot, Version 4.0; San Diego, U.S.A.).

RESULTS

Litter mass loss

In the experiment using litterbags allowing earthworm access, Cortez (1998) observed that the litter mass loss after about 2 yr of decomposition in the field varied from site to site. Beech was the least decomposed litter whatever the site (from 38.5% ± 6.6 to 61.7% ± 8.0) followed by holm oak (from 62.7% ± 8.5 to 70.4% ± 5.7), by sessile oak (from 63.5% ± 8.6 to 84.6% ± 6.5) and by sweet chestnut (from 65.8% ± 7.5 to 88.6% ± 5.7). In our experiment, after the same time of exposure in the field at Anduze, the litter mass loss from sweet chestnut, sessile oak, holm oak and beech were, respectively, 84.6% ± 8.3, 78.6% ± 6.9, 64.8% ± 3.6, 56.5% ± 5.5 in 0.5 cm mesh litterbags and 64.1% ± 5.7, 48.4% ± 7.7, 38.5% ± 9.0, 34.9% ± 5.3 in 0.1 cm mesh litterbags (Table 1). In our experiment the difference (from 20.5% to 30.2% depending on the litter) between the remaining litter in litterbags allowing or preventing earthworm access substantiates the effect of earthworms on litter decomposition although other macrofauna can be also involved in this decomposition.

Effect of earthworms on litter breakdown

Tian *et al.* (1995) quantified the effects of earthworms and millipedes on the breakdown of different plant residues using the formula adapted from Seastedt (1984): $BF = (A - B)/(100 - B) \times 100\%$,

where BF = breakdown of plant residues by the soil fauna, A = % of remaining plant residues without fauna and B = % of remaining plant residues with fauna. BF measures the breakdown of plant residues accounted by soil fauna at a given time. It is not a decomposition rate. Applying this formula to our experiment it is possible to quantify the effect of earthworms on litter decomposition (Table 2). In the first stage of decomposition the effect of earthworms was low (from 2.5% for *F. sylvatica* to 10.3% for *Q. ilex* after 244 d in field) and litter decay was presumably performed by the soil microbiota. The earthworm effect increases with time to reach a maximum after 425 d for *C. sativa* (BF = 37.2%) and *Q. ilex* (BF = 44.6%) and respectively after 549 and 760 d for *Q. petraea* (BF = 43.0%) and for *F. sylvatica* (BF = 38.2%). Table 3 shows the decomposition rates d^{-1} with or without earthworm action in the both experiments at the Anduze site. If it is assumed that earthworm activity was negligible in the first year of the first experiment, the acceleration in the rate of litter decomposition due to the earthworm effect can be estimated. In the first experiment the ratios of the rates (K_{w1}/K_{w2}) varied from 3.6 to 12.5 depending on the type of litter, whereas in the second, they only ranged from 2.6 to 3.8 (K_{w3}/K_{w4}) with a mean of 5.2 over both experiments combined. The difference between the two experiments was rather large for *F. sylvatica* litter but slightly lower for *Q. petraea* litter. This difference could be attributed to the quality of the leaves which were collected at different times of year for each experiment.

DISCUSSION

In the first experiment Cortez (1998) showed that the litters at Le Vernet and Salidès mineralised at a constant decomposition rate while, at Anduze, the litter disappearance rates presented two successive stages. The first was relatively slow (up to 314 to 376 d depending on the litter species) and was followed by a second faster stage (from 314 or 376 d to 769 d in field). It was assumed that the increase of litter decay rates during the second stage was due to the consumption of litter materials by earth-

Table 1. Litter decomposition (mean ± SE) in 0.5 cm mesh litterbags (first experiment) and simultaneously in 0.1 and 0.5 cm mesh litterbags (second experiment) at Anduze after 2 yr exposure in the field

Litter species	Litter decay (% initial weight)		
	First experiment 0.5 cm mesh size	Second experiment 0.1 cm mesh size 0.5 cm mesh size	
<i>C. sativa</i>	88.6 ± 5.7	64.1 ± 5.7	84.6 ± 8.3
<i>Q. petraea</i>	84.6 ± 6.5	48.4 ± 7.7	78.6 ± 6.9
<i>Q. ilex</i>	70.4 ± 5.7	38.5 ± 9.0	64.8 ± 3.6
<i>F. sylvatica</i>	61.7 ± 8.0	34.9 ± 5.3	56.5 ± 5.5

Table 2. Effect of earthworms on the breakdown of the different litters at Anduze

Litter exposure time (d)	<i>Castanea sativa</i>				<i>Quercus ilex</i>				<i>Quercus petraea</i>				<i>Fagus sylvatica</i>			
	Remaining litter (%)		BF (%)		Remaining litter (%)		BF (%)		Remaining litter (%)		BF (%)		Remaining litter (%)		BF (%)	
	-EW (A)	+EW (B)	-EW (A)	+EW (B)	-EW (A)	+EW (B)	-EW (A)	+EW (B)	-EW (A)	+EW (B)	-EW (A)	+EW (B)	-EW (A)	+EW (B)	-EW (A)	+EW (B)
0	100	100	0	0	100	100	0	0	100	100	0	0	100	100	0	0
244	75.8 ± 2.7	74.1 ± 2.0	6.3	6.3	83.2 ± 5.2	81.3 ± 6.3	10.3	10.3	84.6 ± 3.1	83.0 ± 3.2	9.2	9.2	91.0 ± 5.9	90.8 ± 1.3	2.7	2.7
370	73.2 ± 2.9	66.7 ± 3.1	19.5	19.5	77.8 ± 4.8	69.7 ± 5.6	26.6	26.6	81.0 ± 4.2	72.8 ± 5.4	30.2	30.2	91.5 ± 5.1	90.0 ± 2.7	15.1	15.1
425	62.5 ± 4.6	40.2 ± 5.5	37.2	37.2	74.7 ± 6.1	54.3 ± 4.5	44.6	44.6	71.1 ± 4.4	56.5 ± 3.5	33.6	33.6	87.6 ± 3.6	83.8 ± 3.5	23.2	23.2
489	51.9 ± 4.5	32.5 ± 4.9	28.8	28.8	71.3 ± 5.7	51.4 ± 6.5	41.1	41.1	69.1 ± 5.3	53.6 ± 5.2	33.5	33.5	85.3 ± 5.2	81.7 ± 5.2	19.8	19.8
549	46.2 ± 6.8	31.0 ± 6.6	22.0	22.0	66.2 ± 2.5	46.8 ± 6.2	36.4	36.4	66.8 ± 4.8	41.7 ± 4.8	43.0	43.0	78.6 ± 4.5	68.5 ± 7.5	32.0	32.0
760	35.9 ± 5.7	15.4 ± 6.2	24.2	24.2	61.5 ± 3.5	35.2 ± 3.2	40.6	40.6	51.7 ± 7.2	21.4 ± 3.1	38.5	38.5	65.1 ± 6.3	43.6 ± 5.5	38.2	38.2

A = % of remaining litter without fauna (mean ± SE, n = 10).

B = % of remaining litter with fauna (mean ± SE, n = 10).

BF = % of breakdown of litter by soil fauna.

+EW = with earthworms.

-EW = without earthworms.

worms, which were much more abundant at Anduze than at Le Vernet and Salidès (Table 2 of Cortez, 1998). In contrast the slower litter disappearance rates during the first stage of decay could be explained by the fact that litters became more palatable for earthworms only after preliminary microbial decomposition in the soil (Wright, 1972; Cooke and Luxton, 1980; Cooke, 1983; Cortez and Hameed, 1988) which was similar in the two experiments (between 314 and 376 d depending on the experiment and litter species; Fig. 2). In the second experiment the comparison of litter disappearance in litterbags with or without earthworm action (Fig. 2) showed the importance of the faunal effect. This observation confirms that of Kurcheva (1960) who reported in a chestnut forest a 6-fold increase in the rate of decomposition with faunal participation. However, the earthworm effect also depended on the quality of litters and residues and our results agree with those of van Rhee (1963), Satchell and Lowe (1967), Edwards and Heath (1975), Brattsten (1979), Hartenstein (1982), Cortez and Hameed (1988) and Cortez *et al.* (1989) who showed the relationships between chemical composition of litter and food preference of earthworms.

Earthworm activity is roughly proportional to their biomasses but the type of activity depends on ecological categories. Litter decomposition is essentially performed by anecic, epi-anecics and epigeous species which were 10.6 and 11.3 times more abundant at Anduze than at Le Vernet and Salidès respectively. At Anduze litter feeders (anecics + epi-anecics + epigeous) were dominant with large population numbers. Consequently, the decomposition rate was high, leaving a soil with a low organic matter content. Two pedological factors indicate that arid conditions occurred in the topsoil, a low Lang aridity index (Ozenda, 1964) and a coarse soil texture (Cortez, 1998; Table 1). In contrast, the presence of *L. terrestris* indicated permanent soil moisture present in the deeper soil layers as this species only survives in soils where this condition prevails (Bouché, 1985). For this reason the higher earthworm biomass at Anduze was not paradoxical in spite of a relatively dry topsoil because of water accumulation in deeper horizons allowing earthworm survival. In addition *L. terrestris* is well known to bury litter in its burrows before feeding. In fact the high anecic activity leaves a bare soil with practically no litter on it, after the wet winter season. So the litterbags on the topsoil were subjected to a relatively dry microclimate. In contrast, the relatively low litter-feeding activity in the other two sites was linked to an accumulation of organic matter in the topsoil.

We also observed that, at Anduze, there always was a large quantity of casts deposited in the litterbags and we tried to calculate the mineral contamination of litter by earthworm casts. At each

Table 3. Comparison of the decomposition rates d^{-1} (K_w) at Anduze with or without earthworm action in the two experiments

	First experiment			Second experiment		
	$K_{w1} \times 10^{-3}$ (+EW)	$K_{w2} \times 10^{-3}$ (-EW)	K_{w1}/K_{w2}	$K_{w3} \times 10^{-3}$ (+EW)	$K_{w4} \times 10^{-3}$ (-EW)	K_{w3}/K_{w4}
<i>C. sativa</i>	5.13	0.93	5.5	4.12	1.28	3.2
<i>Q. ilex</i>	2.67	0.74	3.6	1.75	0.67	2.6
<i>Q. petraea</i>	3.98	0.59	6.7	3.00	0.80	3.8
<i>F. sylvatica</i>	3.01	0.24	12.5	1.76	0.48	3.7

K_w values were calculated from exponential regression between % of remaining material (in mass) and time (d) according to the following formula: $W = W_0 e^{-K_w t}$.

W = % remaining litter at time t .

W_0 = % mass at time 0.

K_w = decomposition rate d^{-1} .

+EW = with earthworm action.

-EW = without earthworm action.

sampling the linear regressions ($y = ax$) between the percentage litter weight loss (y) (% initial weight) and the percentage of litter C released (x) (% initial C) were highly significant for all litter species combined (for $n = 3$ to 13; Anduze $0.923 < r < 0.985$; Le Vernet $0.902 < r < 0.952$; Salidès $0.895 < r < 0.936$; Table 4). Theoretically, the percentage weight loss (TWL = total weight

loss) would correspond to the percentage of C released (TCL = total carbon loss) in the case where $TWL/TCL = 1$. But in our experiment $0.818 < TWL/TCL < 0.997$ showing that the litter weight loss decreased slower than the C release. This suggests that litters were being contaminated by soil minerals and that the TWL/TCL value could be used as a "contamination" index. The

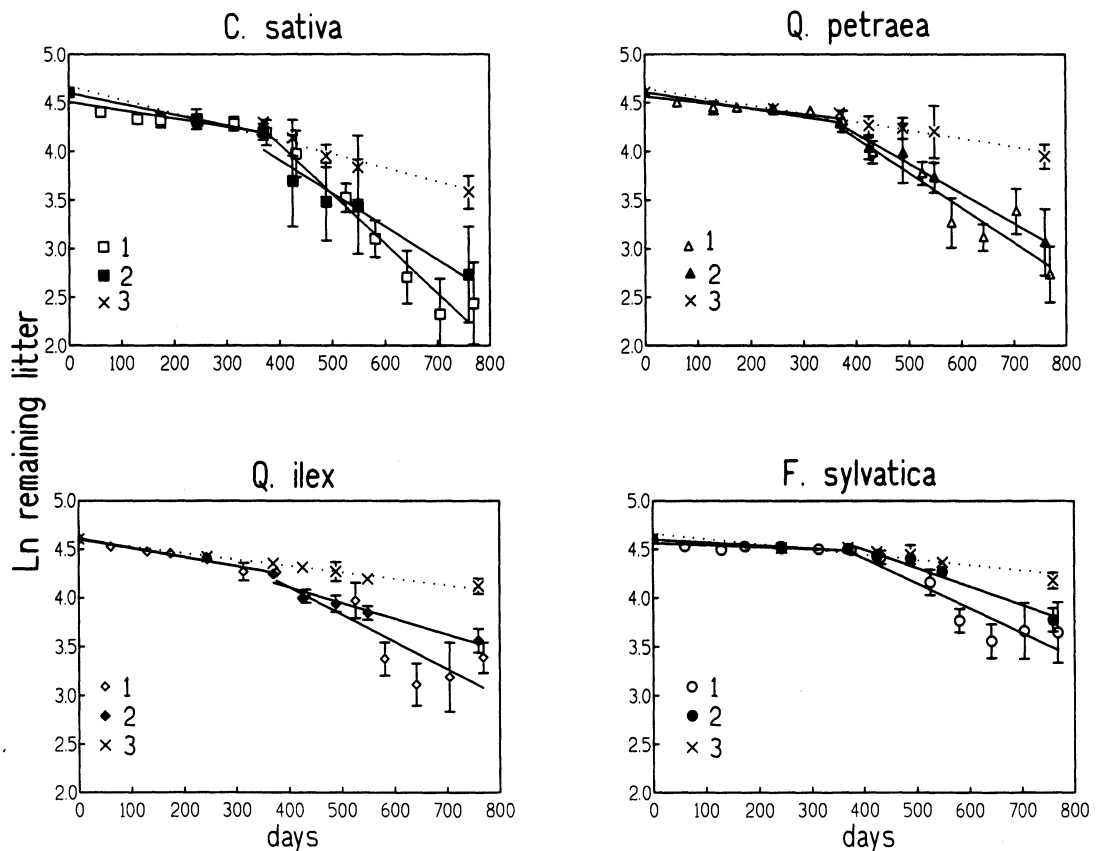


Fig. 2. Disappearance in the field (means \pm SE; $n = 10$) of the different litter species at Anduze in the two experiments. (1) Experiment in litterbags allowing earthworm access (0.5 cm mesh size) (Cortez, 1998); (2) our experiment in litterbags allowing earthworm access (0.5 cm mesh size); (3) our experiment in litterbags preventing earthworm access (0.1 cm mesh size).

Table 4. TWL-to-TCL ratio (\pm SE) calculated with data of the first experiment, all litter species combined

Exposure time in field (d)	n	Anduze		Le Vernet		Salides	
		TWL/TCL	r	TWL/TCL	r	TWL/TCL	r
130	9	0.818 \pm 0.031	0.972	0.997 \pm 0.023	0.903	0.981 \pm 0.022	0.895
174	13	0.825 \pm 0.021	0.923	0.988 \pm 0.014	0.959	0.946 \pm 0.025	0.901
243	17	0.671 \pm 0.039	0.968	0.953 \pm 0.014	0.915	0.907 \pm 0.025	0.902
314	21	0.645 \pm 0.066	0.952	0.918 \pm 0.019	0.902	0.890 \pm 0.016	0.917
376	25	0.667 \pm 0.071	0.965	0.932 \pm 0.014	0.905	0.875 \pm 0.021	0.899
432	29	0.715 \pm 0.064	0.975	0.936 \pm 0.013	0.935	0.872 \pm 0.022	0.921
526	33	0.778 \pm 0.045	0.985	0.946 \pm 0.007	0.936	0.875 \pm 0.026	0.915
581	37	0.835 \pm 0.025	0.972	0.959 \pm 0.004	0.947	0.892 \pm 0.020	0.912
642	41	0.866 \pm 0.018	0.986	0.965 \pm 0.004	0.952	0.902 \pm 0.018	0.928
705	45	0.880 \pm 0.012	0.982	0.963 \pm 0.004	0.945	0.904 \pm 0.021	0.932
769	49	0.897 \pm 0.006	0.981	0.959 \pm 0.004	0.952	0.903 \pm 0.023	0.936

TWL = % total weight loss of litter (as % initial weight).
 TCL = % total carbon loss of litter (in % initial C).
 r = correlation coefficient ($P < 0.05$ for $n = 9$; $P < 0.001$ for $n > 9$).

greater the value of TWL/TCL the more the litter is contaminated by minerals. We thought and recorded that the litters were contaminated by minerals from casts deposited by earthworms. This result is confirmed by the fact that the litter contamination was well related to the biomass of earthworm casting on the soil surface (anecics and epi-anecics) (Le Vernet; minimum TWL/TCL = 0.918; anecic and epi-anecic biomass = 17 g m⁻². Salidès; minimum TWL/TCL = 0.872; anecic and epi-anecic biomass = 15 g m⁻². Anduze; minimum TWL/TCL = 0.645; anecic and epi-anecic biomass = 178 g m⁻²) (Table 2 of Cortez, 1998, Table 4).

The curve plotting the TWL/TCL ratio against time showed a decrease in the ratio corresponding to an increase in mineral deposition in the remaining

litters. In the first experiment with wide (0.5 cm) mesh litterbags the TWL/TCL ratio reached a minimum after 320 d of exposure in field (Fig. 3). After that time the curve increased up to about 700 d and stabilised up to 769 d. The shape of the curve would indicate mineral contamination by casts during the first 320 d of exposure followed by a decrease in this contamination. In the first experiment (Cortez, 1998) we did not suspect that the effect of earthworms on litter decomposition would be so marked. It is only after calculation that we discovered the important changes of TWL/TWC ratios but we had not enough samples to verify the mineral contamination by measurement of ash contents. So, to confirm our hypothesis of a contamination by earthworm casts, we

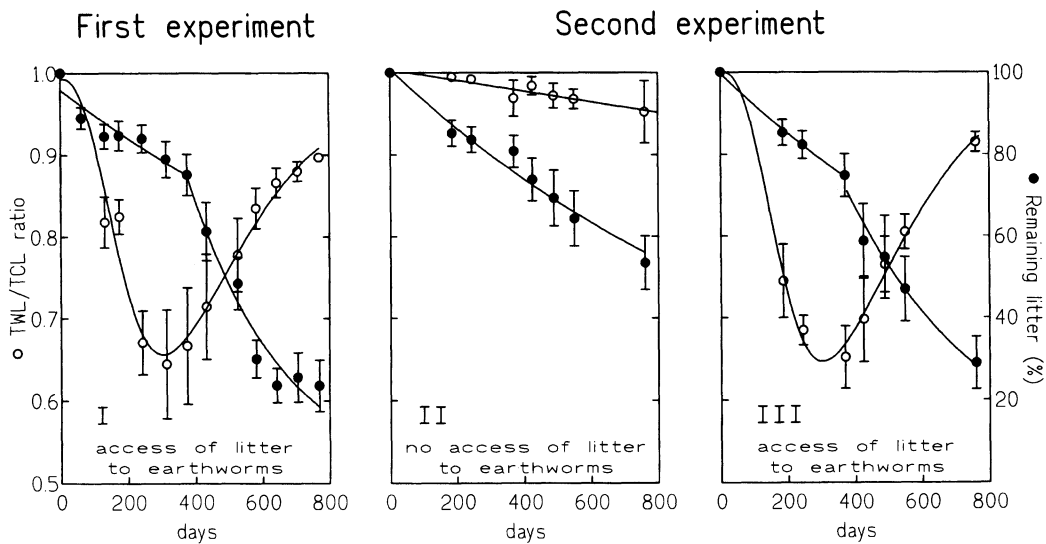


Fig. 3. Relationships (1) TWL-to-TCL ratio (\pm SE) and (2) litter decomposition percentage in terms of time in the two experiments. In the first experiment, only litterbags allowing access of litter to earthworms were used (I) while, in the second experiment, we used simultaneously litterbags allowing (II) and preventing earthworm access to (III). At each sampling time, all litter species have been combined. TWL = % total weight loss of litter (as % initial weight); TCL = % total carbon loss of litter.

Table 5. TWL-to-TCL ratio (\pm SE) calculated with data of the second experiment, all litter species combined

Exposure time in field (d)	<i>n</i>	+EW		-EW	
		TWL/TCL	<i>r</i>	TWL/TCL	<i>r</i>
185	9	0.745 \pm 0.045	0.975	0.995 \pm 0.003	0.986
244	13	0.685 \pm 0.018	0.982	0.992 \pm 0.005	0.990
370	17	0.652 \pm 0.038	0.991	0.969 \pm 0.022	0.978
425	21	0.698 \pm 0.052	0.975	0.984 \pm 0.011	0.972
489	25	0.765 \pm 0.034	0.974	0.972 \pm 0.015	0.983
549	29	0.805 \pm 0.021	0.980	0.968 \pm 0.012	0.962
760	33	0.915 \pm 0.012	0.979	0.952 \pm 0.028	0.985

This experiment was carried out at Anduze in litterbags allowing or preventing the earthworm input.

TWL = % total weight loss of litter (as % initial weight).

TCL = % total carbon loss of litter (in % initial C).

r = correlation coefficient ($P < 0.001$).

+EW = with earthworms.

-EW = without earthworms.

systematically measured ash contents in all the samples from the second experiment.

The second experiment with small 0.1 cm and wide 0.5 cm mesh litterbags confirmed the results of the first experiment. When earthworms were allowed access to litter, the TWL/TWC ratios varied from 0.652 to 0.915 and hence the shape of the curve of relative contamination by casts was similar to that obtained in the first experiment. In contrast when litter was protected by smaller mesh-size litterbags the TWL/TWC ratios varied from 0.952 to 0.995 (Table 5) indicating only a very weak mineral contamination. The results showed that the TWL/TWC ratios and the ash content percentages (Table 6) evolved in the same way reaching a maximum of contamination between 370 and 489 d exposure in field with earthworms. So the ash content percentages varied from 7.5% to 9.6% while they did not exceed 2.1% without earthworms. Then we can assume that, during the first stage of decomposition, the litters were ploughed in by casts involving an enhancement of microbial activity and preliminary litter microbial decomposition.

Furthermore the pH of casts (6.1) exceeded the pH of the A₀ horizon (5.3) by 0.8 pH units indicating that earthworm casts spread cations onto the topsoil. This cation flux enhances both the pH and the preliminary litter decomposition and makes the litters more palatable for earthworms, increasing the litter decomposition rate observed in the second and faster stage of decay. So the litter ploughing-in acts on microbial decomposition both by microclimatic sheltering and by changes in chemical conditions. In the two experiments the TWL/TCL ratio increased after 320 and 370 d of exposure respectively, indicating a decrease in mineral contamination. As yet we cannot produce a completely convincing explanation of this result but two hypotheses could be put forward.

1. Leaching by rainfall could remove the minerals deposited on litter.
2. Earthworms could feed on litter leaf blades rather than litter petioles, stems or ribs, thus decreasing the area for mineral deposition, and hence the remaining litter would appear relatively less contaminated.

Table 6. Ash content (\pm SE) calculated with data of the second experiment, all litter species combined

Exposure time in field (days)	<i>n</i>	+EW	-EW
		ash content (%)	ash content (%)
185	40	4.2 \pm 0.8	1.4 \pm 0.3
244	40	6.5 \pm 1.5	1.6 \pm 0.4
370	40	9.6 \pm 2.0	1.3 \pm 0.6
425	40	7.5 \pm 2.1	1.7 \pm 0.4
489	40	8.2 \pm 2.1	1.7 \pm 0.7
549	40	4.2 \pm 0.7	1.8 \pm 0.2
760	40	2.6 \pm 0.8	2.1 \pm 0.3

This experiment was carried out at Anduze in litterbags allowing or preventing the earthworm input.

+EW = with earthworms.

-EW = without earthworms.

The simple measure of the litter weight loss and the litter C content and the method of calculation applied enabled us to clearly demonstrate the contamination of the litter by earthworm casts. However if the litter sample quantities are not limited it will be faster to measure the same change in litter content by a systematic ash content analysis.

CONCLUSIONS

The results obtained in the first experiment using the litterbags method led us to propose a model that takes into account the mechanical activity of earthworms in the process of decomposition. We then concentrated our study on the importance of ploughing-in by earthworm casts in the process of forest litter decomposition. As was stated in the introduction, the importance of worm casts in ecosystems was observed and measured a long time ago (Darwin, 1837; Stöckli, 1928), but there are no studies quantifying the effects of the phenomenon on the decomposition process. On the basis of the results of these two experiments, we have shown that the effects of earthworms, by changing the surrounding conditions for litter decomposition, on average accelerated the decomposition of the litter species studied by a factor of about 5, with the exception of the observations on *F. sylvatica* which proved to be rather variable.

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