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Effect of climate, soil type and earthworm activity on nitrogen transfer from a nitrogen-15-labelled decomposing material under field conditions

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Abstract N transfer from ^{15}N-labelled decomposing material into the microbial biomass and inorganic N forms was studied for more than 2 years at three experimental sites differing in climatic conditions and earthworm abundance. The ^{15}N-labelled decomposing material was mixed with low-elevation soil (LES), mid-elevation soil (MES) and high-elevation soil (HES). The amended soils were put into two kinds of plastic cylinders closed on both sides with nets preventing (0.1 cm mesh) and allowing (0.5 cm mesh) access by earthworms, and were buried in soil (20 cm depth) to monitor the transfer of N from the ^{15}N-labelled decomposing material. Climate and soil type play an important role in the release of N from decomposing material. LES transplanted to more humid sites (mid- and high-elevation sites) showed an increase in most of its biological activities (N atom % excess, and microbial biomass C and N). Furthermore, LES was a sandy soil in which the ^{15}N-labelled decomposing material was less bound than in MES and HES, which contained more silt and clay. This resulted in faster organic matter turnover when climatic conditions were favourable. The presence of earthworms greatly increased the quantity of inorganic N (mainly NH_4^+) in the soils and enhanced the release of N from the ^{15}N-labelled decomposing material and the native organic matter, compared to soil without earthworms.

Key words Nitrogen-15 atom percent excess · Field nitrogen transfer · Microbial biomass carbon and nitrogen · Inorganic nitrogen · Earthworm activity

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Introduction

One of the fundamental problems of sustainable development concerns the conservation and management of renewable resources. The balance of these resources depends on the requirements of plants and also on soil biological functions. The nutrients essential for plant growth are generally tied up in the soil, and their availability to plants depends on the functioning of vegetation, on environmental factors and on soil biota, of which the soil fauna is an essential component. Earthworms, in particular, have been shown to play critical roles in soil functions. They increase soil stability and soil infiltration rates (Ehlers 1975; Roth and Joschko 1991; Bouché and Al-Addan 1997) and play a role in the P and N cycles (Sharpley et al. 1979; Syers et al. 1979; Scheu 1987; Cortez and Bouché 1987; Hameed et al. 1994; Bouché et al. 1997). Likewise, earthworms have been shown to enhance plant productivity (Stockdill and Cossens 1969; Edwards and Lofty 1978; Ruz-Jerez et al. 1992) and litter decomposition rates (Scheu and Wolters 1991; Cortez 1998; Cortez and Bouché 1998). However, little attention has been paid to the effect of climate, soil type and earthworm activity on pre-decomposed litters incorporated into the soil. In forest ecosystems the humus is as significant a component as the litter with respect to nutrient turnover because most of the soil organic N is present in humified forms (Prévot 1970). N is closely bound to humus compounds, and the mineralization rates of these relatively stable compounds are low (Stevenson 1986). However, indirect evidence suggests that at least part of this N in the form of lignin-NH_4^+, quinone-NH_4^+ or carbohydrate-amino acid condensation products (Parsons and Tinsley 1975; Stevenson 1982) is available to plant roots and micro-organisms (Ross 1988). To study the movement of N from decomposing material into the microbial biomass and inorganic forms, uniformly ^{15}N-labelled material has to be used in order to differentiate between the N from native organic matter in the soil and that of added organic material. Ideally, pre-decomposed ^{15}N-la-

belled litters should be incorporated into the soil, but it is technically difficult to obtain uniformly ^{15}N-labelled litters from tree leaves. We therefore chose to use a standard plant material that was uniformly labelled with ^{15}N and which was subjected to a preliminary decomposition. The preparation of this ^{15}N-labelled material was based on the work of Bottner et al. (1998), who showed that the decomposition of wheat straw in soils incubated at a controlled temperature and humidity occurs at different rates in two stages: a fast initial stage (0–50 days of incubation) followed by a slower stage (>50 days of incubation) which involves the decomposition of partially humified organic matter. For this reason, in the current experiment, we used ^{15}N-labelled humified organic matter corresponding to this second decomposition stage and prepared from ^{15}N-labelled wheat straw. While this material is not identical to humus originating from forest litters we felt it was adequate analogue with which to test the effects of: (1) climate, (2) soil type and (3) earthworm activity on N release from humified material.

Materials and methods

Experimental sites and soil characteristics

The three experimental sites were situated in chestnut (*Castanea sativa* Mill.) forests of the Cevennes highlands (south of France) at different altitudes and contrasting climates. At each site, an automatic meteorological station continuously monitored meteorological variables (air temperature and humidity, wind speed). The soil temperature was continuously recorded. Soil moisture in the top 10 cm of soil was measured weekly by a dry weight method. The characteristics of the sites and the soils are shown in Table 1.

Soil preparation

Soil from low-, mid- and high-elevation sites were collected from the upper 5 cm of the A_1 horizon in different areas of each site, air-dried for a few days, sieved (2 mm) and homogenized.

Preparation of ^{15}N-labelled composted straw and amendment of soils

About 1200 g leaves and stems from uniformly ^{15}N-labelled mature wheat straw was cut into pieces (4–5 cm), mixed with 1500 g sieved soil (2 mm), moistened to 80% water-holding capacity (WHC) and left to decompose for 60 days at 28 °C. The soil water content was controlled by weighing every 4 to 5 days, and readjusted if necessary. After the 60-day decomposition period, the mixture of composted straw and soil was suspended in water, stirred and the soil separated by sedimentation. This operation was repeated several times until all the soil particles had been removed. The supernatant was then passed through a 2-mm sieve that retained plant debris composed of about 80% straw particles <0.5 cm in size and 20% residues of 0.5–5 cm; bigger particles were carefully cut into smaller pieces (0.5 cm) and added to the other debris. This mixture of organic matter (about 850 g ^{15}N-labelled composted straw) was used as a supplement. The blackish colour and friable consistency of the debris, in view of the results of Bottner et al. (1998), lead us to think that this organic matter was in the second decomposition stage, and indicated the decomposition of partially humified organic matter. All this material was dried at 60 °C in an oven for 48 h before soil amendment. Ninety six units of each soil [low-elevation soil (LES), mid-elevation soil (MES) and high-elevation soil (HES); 260 g air-dried] were amended with 1129 mg, 885 mg, and 1522 mg, respectively, of the ^{15}N-labelled decomposing material (C 39.2%, N 1.2%, atom % excess ^{15}N 9.53%); each amendment corresponded to 5% soil C. The ^{15}N-labelled decomposing material was mixed into the soil samples for 20 min. The mixtures were remoistened to 80% WHC and put into plastic cylinders (4 cm height, 10 cm diameter, 0.4 cm wall thickness) closed at both ends as follows:

1. To prevent earthworm access into the cylinders, 216 cylinders were closed with 0.1-cm mesh nets.
2. To allow earthworm access into the cylinders, 72 cylinders were closed with 0.5-cm mesh nets.

Then all the cylinders were frozen and transported into the field in a freezing compartment to limit microbial activity and to avoid loss of the cylinder contents.

Experimental procedure

Ninety-six cylinders were buried at random horizontally into each site (20 cm depth) in 12 small quadrats (each containing eight cylinders) and left to decompose for 848 days at the low-, mid- and high-elevation sites. The treatments were as follows. Treatments 1–4 at the low-elevation site: LES plus earthworms (+ew; 1), LES minus earthworms (–ew; 2), MES-ew (3), HES-ew (4). Treatments 5–8 at the mid-elevation site: MES + ew (5), MES-ew (6), LES-ew (7), HES-ew (8). Treatments 9–12 at the high-elevation site: HES + ew (9), HES-ew (10), LES-ew (11), MES-ew (12).The cylinders were sampled approximately every 3 months as indicated in Table 2, and the recovered material was analysed.

The comparison of the results between treatments 1 and 2, 5 and 6 and 9 and 10 indicated the effect of earthworms on the decomposition of the humus analogue in each soil under the climatic conditions of each site.

The comparison of the results between treatments 2, 7, 11; 3, 6, 12 and 4, 8, 10 indicated the effect of climate on the decomposition of the humus-analogue in each soil.

The comparison of the results between treatments 2, 3, 4; 6, 7, 8 and 10, 11, 12 indicated the effect of soil type on the decompositionof the humus-analogue.

Analytical methods

Soil microbial biomass C (MB-C) was determined by the fumigation-extraction method (Vance et al. 1987) using a K_c factor of 0.38. Soil MB-N was determined by the method of Brookes et al. (1985) using a K_n factor of 0.54. Total N was determined by the Kjeldahl method. Total inorganic N (TIN; 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^+ was first distilled by steam distillation, then NO_3^- was distilled after addition of Devarda's mixture. NH_4^+ and NO_3^- were analysed separately by colorimetry flow analysis. Then all ^{15}N-labelled distillates 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 NH_4^+ salts by oxidation with lithium hypobromite (Rittenberg 1948). N_2 enrichments were determined by optical spectrometry (Guiraud and Fardeau 1980). It was not necessary to measure the ^{15}N remaining in the soil in relation to time, since it was very difficult to make an overall ^{15}N balance. Losses through leaching, root uptake, denitrification, etc. are, in practice, almost impossible to estimate in the field.

Statistical analysis

Statistical analyses were performed with STATITCF software. *t*-Tests were used for comparison of means and ANOVA to assess: (1) the interaction between soil types and climate, and (2) the sig-

nificance of differences between NH_4^+-N atom % excess ^{15}N (NH_4^+-E%) and MB(-E%) with or without earthworms.

Results

Earthworm biomass

The total earthworm biomass was determined 4 times (March and July 1994, October and December 1995), and the long-term average varied from 6% to 10% for each site. However, the total earthworm biomass differed greatly between the three sites, from 182 g fresh weight m^{-2} (93.4% anecics, 4.4% epianecics, 1.6% epigeous, 0.6% endogeous) at the low-elevation site, to 17 g fresh weight m^{-2} (100% anecics) at the mid-elevation site and 29 g fresh weight m^{-2} (51.7% anecics, 3.4% epigeous, 44.8% endogeous) at the high-elevation site (Cortez and Bouché 1998). The decomposition of the labelled straw material, which was buried in the soil, was essentially performed by anecic, epianecic and endogeous species. These three ecological categories of earthworms were 11 and 6.7 times more abundant at the low- than at the mid- and high-elevation sites.

MB-C, MB-N and MB-E%

The temporal changes in MB-C, MB-N and MB-E% o LES-ew were similar at each site (Fig. 1) with high val ues initially, which then, except for MB-N, usually de creased slowly until the end of the experiment. The MB-C, MB-N and MB-E% of LES were, on average about 20% lower when this soil was incubated at the low- rather than at the mid- or high-elevation sites.

The MB-C of the MES at the low- and high-eleva tion sites, was, on average, 7% and 9% lower, respec tively, than at the mid-elevation site, while MB-N re mained unchanged at all sites. MB-E% was generally the same at the low- and the mid-elevation site and 9% lower at the high-elevation site (Fig. 1). It is also note worthy that all measured soil activities were often low er at the low-elevation site (Fig. 1).

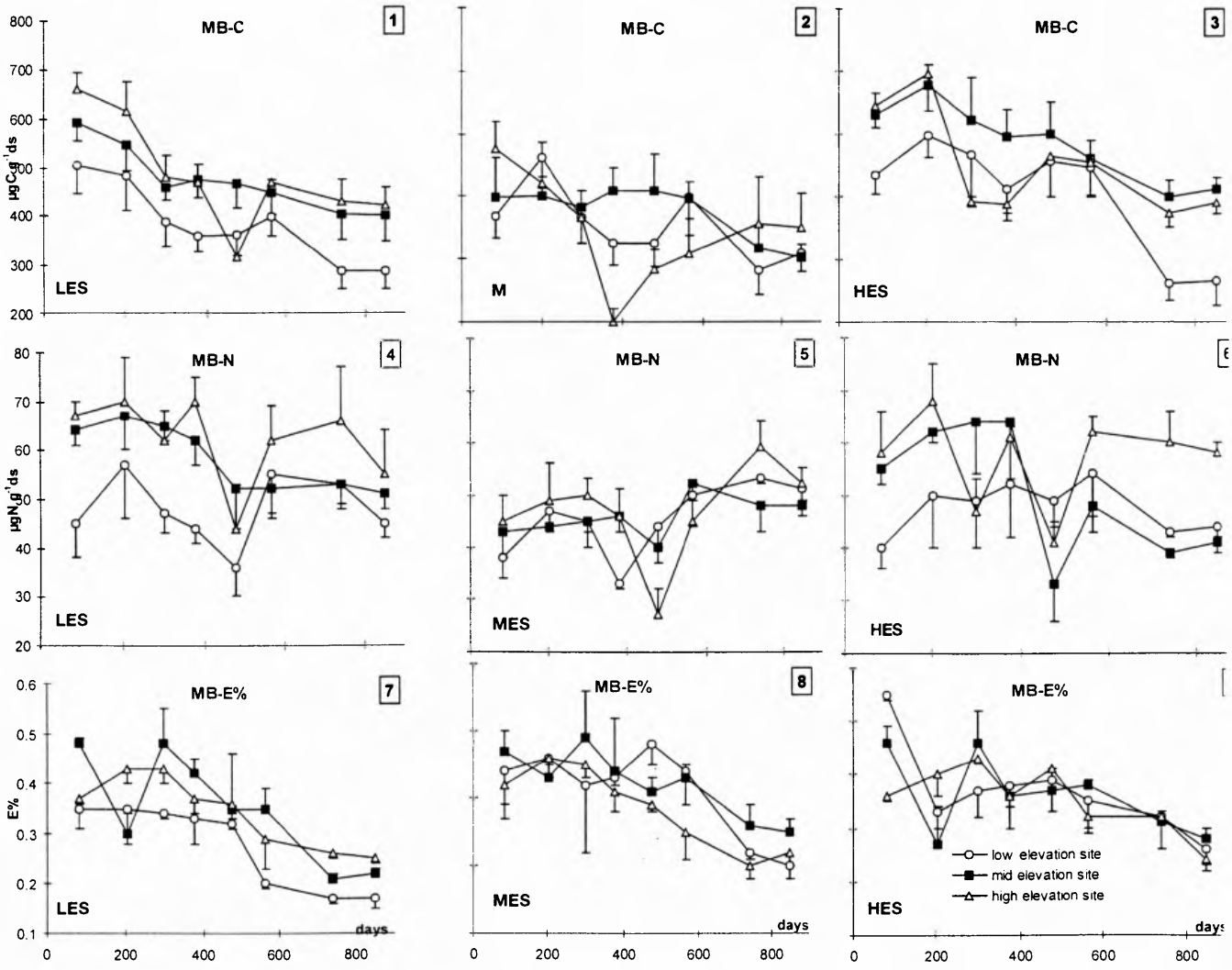


Fig. 1 Changes in microbial biomass C (*MB-C*), MB-N and MI atom % excess ^{15}N (*MB-E%*) during an 848-day incubation in th field at the three sites (means ± SE, $n = 3$). *LES* low-elevation site *MES* mid-elevation site, *HES* high-elevation site, *ds* dry soil

In cylinders of LES allowing earthworm access, the MB-C reached a mean value of 499 μg C g^{-1} dry soil by day 595, and the MB-N reached a value of 61 μg N g^{-1} dry soil by day 377, compared to only 415 μg C g^{-1} dry soil and 46 μg N g^{-1} dry soil, respectively, for LES-ew (Fig. 2). However, there were no differences between the levels of MB-E% in cylinders with or without earthworms (Table 3). The results indicated an enhancement of the MB in treatments with earthworms, without greater consumption of labelled material. There were no significant differences between MES and HES treatments with or without earthworms (Fig. 2, Table 3).

Inorganic N

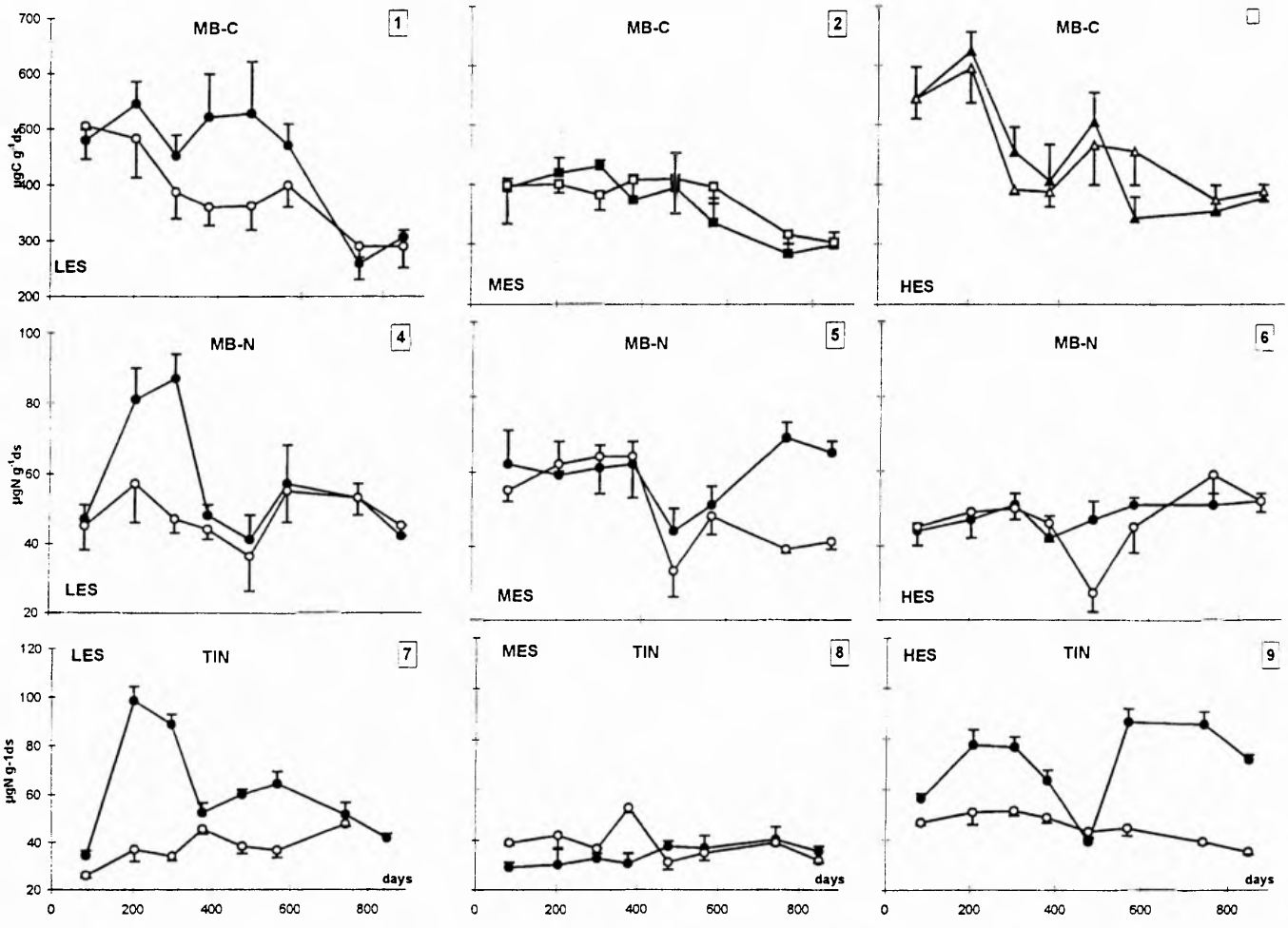
Figures 2 and 3 show the effect of earthworms on the TIN content of LES and HES. During 848 days of field exposure with earthworms, the TIN of LES changed from 34.3 to 41.4 μg N g^{-1} dry soil, and reached two maxima (98.3 and 64.1 μg N g^{-1} dry soil, after 203 days and 595 days, respectively). HES showed, on average, higher values than LES, and also showed two maxima at the same times as LES (77.6 and 86.9 N μg^{-1} dry soil). However, without earthworms, the mean TIN of these two soils did not exceed 37.6 (LES) and 45.1 N μg^{-1} dry soil (HES). The mid elevation site contained few earthworms, hence MES did not show these differences. It is noteworthy that the quantities of NH_4^+ found were very much higher when earthworms were present (Fig. 3). These results confirmed those of various authors who have reported the importance of earthworms in N cycling (Needham 1957; Andersen 1983; Hameed et al. 1994; Bohlen and Edwards 1995; Bouché et al. 1997).

Discussion

Role of soil climate and soil type on microbial biomass and decomposition of the humus analogue

All the measured variables were influenced by both climate and soil type. LES was the soil most influenced by the climatic differences (Fig. 1). When LES was incubated under more humid conditions in the higher sites, it showed average gains of between 20% and 30% for

Fig. 2 Changes in MB-C, MB-N and total inorganic N (*TIN*) in the presence (*filled symbols*) and in absence (*open symbols*) of earthworms during an 848-day incubation in the field at the three sites (means ± SE, $n = 3$). For other abbrevations, see Fig. 1



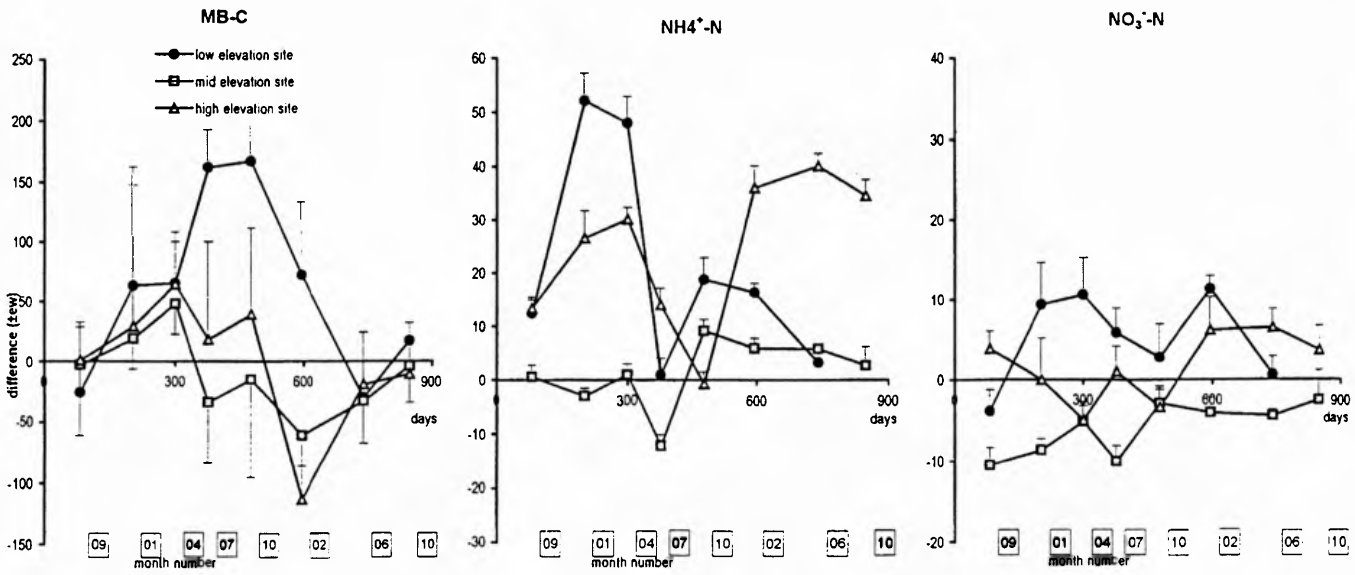


Fig. 3 Differences between MB-C, NH_4^+-N and NO_3^--N ($\mu g\ g^{-1}$ ds) in the presence and absence of earthworms ($\pm ew$) during an 848-day incubation in the field at the three sites. *Numbers in squares* indicate the month of sampling

MB-C and MB-N, respectively. An increase in MB-E% was recorded when LES was incubated at the other sites but this was not the case with MES and HES. This showed that when LES, originating from a drier climate, was transferred to moister sites, the decomposition of the added labelled material was stimulated compared to the native organic material in the soil. In the same way, the MB-C and MB-N of MES and HES were lower when these soils were transplanted to the low-elevation site. The results of these field experiments were consistent with those of a previous study that showed that soil drying resulted in a large decrease in total MB-C and MB-N (Cortez 1989). It is known that the decomposition of organic matter is related to soil microbial activity, to the quality of organic matter, its chemical structure, and also to its binding to organic compounds and clays. After several months of experiment it was possible that the ^{15}N-labelled humus analogue added to the soil was closely bound to mineral components of the soil. Generally, most of the C and N compounds in soil linked to silt- (20–40%) and clay-size (35–70%) fractions (Anderson et al. 1981; Schnitzer and Ivarson 1982; Christensen 1985; Cortez and Cherqui 1991) are strongly aromatic and become resistant to biodegradation (Anderson et al. 1981). LES is a sandy soil (76% sand, 15.5% silt and only 8.5% clay) in which less ^{15}N-labelled organic matter is likely to be bound than in MES and HES, which contain much more silt and clay (Table 1). This could result in faster organic matter turnover in LES when climatic conditions are favourable. So, when microbial activity was not enhanced by earthworm activity, abiotic factors probably controlled the microbial decomposition of the humus analogue. Soil moisture is generally the limiting abiotic factor, but organic matter decomposition depends on soil type too.

Table 4 shows that under the low-elevation and mid-elevation climates the added organic matter decomposed to a greater extent in MES [total ^{15}N (MB plus TIN) as a percentage of initial ^{15}N ($^{15}N/^{15}N$ ini 17.3% and 18.2%, respectively] than in HES ($^{15}N/^{15}N$ ini, only 12.6% and 13.4%, respectively), despite its lower microbial biomass. However, the decompostion of this organic matter in LES was much higher than in the other soils, since on average the LES incorporated 22% of

Table 1 Climate of the sites and physical and chemical properties of the soils (5 cm)

Sites	Altitude (m)	Annual mean rainfall (mm) (1)	Annual mean temp (°C) (2)	Lang aridity index (1)/(2)	Soils						
					pH (H_2O)	Organic C (%)	Total N (%)	C:N ratio	Clay (%)	Silt (%)	Sand (%)
Low elevation	380	1212	11.9	101	5.3	3.79	0.22	17	8.5	15.5	76.0
Mid elevation	520	1850	12.8	148	5.4	2.97	0.16	19	21.5	39.3	39.2
High elevation	860	2355	8.1	261	4.8	5.11	0.34	15	29.9	33.7	36.4

[a] Lower values indicate higher aridity (Ozenda 1964)

Table 2 Experimental procedure for sampling ($n=3$ for each treatment). *LES* low-elevation soil, *MES* mid-elevation soil, *HES* high-elevation soil, *+ew* with earthworms (0.5 cm mesh nets), *−ew* without earthworms (0.1 cm mesh nets)

Treatment	Low-elevation site	Treatment	Mid-elevation site	Treatment	High-elevation site
1	LES (+ew)	5	MES (+ew)	9	HES (+ew)
2	LES (−ew)	6	MES (−ew)	10	HES (−ew)
3	MES (−ew)	7	LES (−ew)	11	LES (−ew)
4	HES (−ew)	8	HES (−ew)	12	MES (−ew)

Table 3 Temporal changes in microbial biomass atom % excess ^{15}N (*MB-E%*) and NH_4^+−N atom % excess ^{15}N (*NH_4^+-E%*) during days at the three field sites. ANOVA on the effects of +ew and −ew on MB-E% and NH_4^+-E%. For other abbreviations, see Table 2

Exposure time (days)		Low-elevation site		Mid-elevation site		High-elevation site	
		MB-E%	NH_4^+-E%	MB-E%	NH_4^+-E%	MB-E%	NH_4^+-E%
83	(+ew)	0.35	0.30	0.37	0.31	0.43	0.35
	(−ew)	0.35	0.33	0.37	0.32	0.36	0.31
203	(+ew)	0.36	0.27	0.37	0.31	0.46	0.33
	(−ew)	0.35	0.33	0.33	0.29	0.40	0.36
300	(+ew)	0.36	0.27	0.40	0.32	0.49	0.31
	(−ew)	0.34	0.33	0.39	0.32	0.43	0.40
377	(+ew)	0.36	0.31	0.38	0.28	0.42	0.32
	(−ew)	0.33	0.31	0.34	0.26	0.36	0.34
476	(+ew)	0.28	0.20	0.29	0.24	0.41	0.31
	(−ew)	0.32	0.25	0.31	0.25	0.41	0.32
595	[+ew]	0.26	0.18	0.30	0.24	0.34	0.29
	(−ew)	0.20	0.17	0.33	0.27	0.32	0.31
741	(+ew)	0.21	0.15	0.27	0.21	0.32	0.22
	(−ew)	0.17	0.15	0.26	0.21	0.32	0.28
848	(+ew)	0.19	0.14	0.27	0.18	0.27	0.21
	(−ew)	0.17	0.14	0.25	0.17	0.24	0.23
ANOVA	ew	1.60^{ns}	10.84^{**}	0.41^{ns}	0^{ns}	22.57^{**}	9.34^{**}
	Time	13.47^{**}	62.06^{**}	10.00^{**}	22.80^{**}	37.07^{**}	10.18^{**}
	ew × time	0.47^{ns}	2.36^{ns}	0.59^{ns}	0.62^{ns}	1.81^{ns}	1.87^{ns}

$^{**}P<0.01$; $^{*}P<0.05$; *ns* non significant

the initial ^{15}N into MB plus TIN when it was transferred to more favourable climates, compared to a mean of 17% for MES and 13% for HES. This enhanced decomposition of the humus analogue in MES was not linked exclusively to the microflora composition, i.e. fungi and bacteria, because the microbial C/N ratios were very similar whatever the soil. However, Bottner et al. (1998) indicated in another study that the main decomposition variables were highly correlated with soil pH, and Cortez and Bouché (1998) reported that an increase in litter pH due to earthworm casting resulted in faster decomposition of litters. The pH of MES (5.4) was higher than that of HES (4.8) and could explain the greater decomposition of ^{15}N-labelled material in this soil.

Effect of earthworms on changes in the decomposition of the humus analogue, microbial biomass and inorganic N

Table 5 shows that the consumption of ^{15}N-labelled organic matter was significantly stimulated in the presence of earthworms at the low- and the high-elevation sites. The mean difference between $^{15}N/^{15}N$ ini in the presence and absence of earthworms was 39% more at the low- compared to the high-elevation site. It seemed likely that this difference between the two sites was related to the much higher earthworm biomass at the low-elevation site compared to the high-elevation site. This hypothesis was also confirmed by the fact that at the mid-elevation site, where the earthworm biomass was lower, there was no increase in the consumption of ^{15}N- labelled material in the cylinders allowing access by earthworms. The earthworm biomass could act: (1) by assimilating the organic matter directly, or (2) by stimulating and increasing the microbial biomass (particularly at the low-elevation site; Fig. 2). However, at the low-elevation site there was no significant difference between MB-E% (Table 3) with and without earthworms, indicating that the ^{15}N-labelled humus analogue was decomposed to a similar extent with earthworms as was the native organic matter. In contrast, at the high-elevation site, a significant increase in MB-E% was recorded, especially in the first year (Ta-

Table 4 ANOVA (−ew) on the effect of climate and soil types (means, $n=21$) on MB-C, MB-N, NH_4^+-N, MB-E%, total inorganic N atom % excess ^{15}N (*TIN-E*%) and total ^{15}N (MB+TIN) as % of initial ^{15}N ($^{15}N/^{15}N$ ini). *LEC* low-elevation climate, *MEC* mid-elevation climate, *HEC* high-elevation climate; for other abbreviations, see Tables 2 and 3

Variables	Climate	Soil type			Soil	Climate	Soil × climate
		LES	MES	HES			
MB-C	LEC	397^{bc}	361^{cd}	425^{b}	**	**	**
	MEC	484^{a}	387^{bc}	498^{a}			
	HEC	491^{a}	345^{d}	461^{a}			
MB-N	LEC	48^{d}	44^{d}	48^{d}	**	**	**
	MEC	59^{b}	46^{d}	52^{c}			
	HEC	63^{a}	46^{d}	58^{b}			
$N\text{-}NH_4^+$	LEC	23.5^{g}	29.1^{e}	30.7^{d}	**	**	**
	MEC	36.7^{b}	24.8^{f}	29.5^{e}			
	HEC	45.3^{a}	21.9^{h}	33.1^{c}			
MB-E%	LEC	0.29^{d}	0.33^{c}	0.38^{a}	**	*	**
	MEC	0.37^{a}	0.33^{bc}	0.37^{a}			
	HEC	0.36^{ab}	0.30^{d}	0.37^{a}			
TIN-E%	LEC	0.27^{de}	0.27^{cde}	0.30^{bc}	**	*	**
	MEC	0.31^{ab}	0.27^{cde}	0.31^{ab}			
	HEC	0.29^{bcd}	0.26^{e}	0.33^{a}			
$^{15}N/^{15}N$ ini	LEC	12.2^{e}	17.3^{b}	12.6^{de}	**	**	**
	MEC	21.7^{a}	18.2^{b}	13.4^{cd}			
	HEC	22.5^{a}	14.3^{c}	14.3^{c}			

$^{**}P<0.01$; $^{*}P<0.05$

$^{a-d}$Means followed by the *same letter* for the same measurement did not differ significantly

ble 3), which indicated an increase in the proportion of the ^{15}N-labelled humus analogue that was used by the soil MB. At the high-elevation site, endogeous species accounted for 44.8% of the earthworm biomass. These species are known to account for considerable microbial activity in the drilosphere because of their extremely high consumption of soil, which passes through their gut (Martin and Lavelle 1992). In the first year of the experiment, the MB that was thus stimulated used the ^{15}N-labelled humus analogue to a greater extent than the native soil organic matter, because it was less resistant. This phenomenon gradually waned with time as the organic matter supplied became increasingly humified.

The presence of earthworms at the low- and high-elevation sites led to a significant increase in the recorded quantities of NH_4^+ (Fig. 3). This increase could have been related either to increased N production or to decreased N losses. Abiotic factors, such as humidity, temperature and leaching, would have led to the same losses of N at any one site since they were the same whether earthworms were present or not. Losses of N from denitrification could, however, have differed in the presence or absence of earthworms, as they release easily mineralisable organic substances (Lee 1983; Martin et al. 1987; Cortez and Bouché 1987) which can stimulate denitrification by decreasing the level of O_2 available in the soil and increasing the level of electron-donor substances (Paul and Clark 1989). If denitrification did occur in these soils, it could only have been increased by the presence of earthworms. The soil moisture content at any sampling time only differed by, at most, 2% between the cylinders with a large or small mesh. Therefore, the quantities of NH_4^+ found in cylinders with a large or small mesh at any given site could be compared since the cylinders were subject to the same environmental constraints (leaching, uptake by roots, etc.), and the N losses would therefore have been the same. Therefore, when a greater quantity of NH_4^+ was found in one of the two systems, it could be assumed that the quantities of NH_4^+ produced were greater. The quantities of NH_4^+ thus found varied from

Table 5 $^{15}N/^{15}N$ ini of the three soils at their sites of origin, (+ew) and (−ew). For abbreviations, see Tables 2, 3 and 4

Soil at site of origin	Exposure time (days)																							
	83			203			300			377			476			595			741			848		
	(+ew)	(−ew)	*t*-test	(+ew)	(−ew)	*t*-test	(+ew)	(−ew)	*t*-test	(+ew)	(−ew)	*t*-test	(+ew)	(−ew)	*t*-test	(+ew)	(−ew)	*t*-test	(+ew)	(−ew)	*t*-test	(+ew)	(−ew)	*t*-test
LES	14.63	13.1	ns	26.40	15.70	**	23.34	14.56	**	17.97	14.57	**	12.87	10.57	**	12.76	8.73	**	10.60	8.43	**	8.45	6.31	*
MES	17.53	19.55	ns	18.36	18.26	ns	21.06	20.32	ns	19.18	19.85	ns	15.56	13.77	ns	16.85	18.08	ns	15.50	14.16	ns	13.22	13.12	ns
HES	19.74	15.12	**	22.42	17.81	*	19.28	15.73	*	18.30	15.36	*	12.07	12.19	ns	17.12	13.03	**	16.35	11.69	**	13.21	10.52	*

$^{**}P<0.01$; $^{*}P<0.05$

one site to another and changed from season to season (Fig. 3). Furthermore, the lowest values of surplus NH_4^+ were found in summer, the season when earthworms (and especially anecics) aestivate. The stimulation of NH_4^+ production could have been related either to an increase in microbial activity due to an earthworm effect, or to earthworm metabolism itself. Both of these hypotheses were examined:

Hypothesis I. Increased NH_4^+ production related to increased microbial activity

The MB increased at the low-elevation site, but there was no synchronisation between its increase and the greater quantity of NH_4^+ found. In contrast, at the mid- and high-elevation sites there was no significant increase in the MB (Fig. 3). It was, however, likely that the earthworms could have stimulated net N mineralisation, resulting in increased TIN production, without there necessarily being any increase in the microbial population. In fact, the increase in net N mineralisation could generally be explained by a qualitative change in the organic compounds being metabolised (decrease in the C/N ratio) and/or by faster microbial turnover resulting from a more active microbial population. If the accumulation of NH_4^+ was related to a stimulation of microbial activity, a NH_4^+-E% similar to the MB-E% that produced it should have been observed, since there was a rapid turnover of the NH_4^+ fraction. This was not, however, the case, as NH_4^+-E% was always lower than E%-MB (Table 3). To better describe this phenomenon, we calculated the degree of isotope dilution (MB-E%/NH_4^+-E%) between the MB and the NH_4^+ found in the soil in the presence and absence of earthworms (Table 6). The mid-elevation site with its low earthworm population (17 g m^{-2}) showed almost identical values for these isotope dilutions in the presence and absence of earthworms. In contrast, at the low- and high-elevation site, significant increases in these values were recorded, especially during periods of NH_4^+ accumulation (Fig. 3). This indicated that there was a significant decrease in NH_4^+-E% compared to MB-E% in the presence of earthworms that could be explained by: (1) a low rate of turnover of the NH_4^+ fraction, or (2) by the supply of NH_4^+ from outside sources (mesofauna, aeolian and rainfall inputs). The rate of turnover of NH_4^+ is related to the rate at which it is used by the microflora (immobilization, nitrification) and by plants. It therefore also depends on environmental conditions which regulate biological activity, but these were the same in systems with and without earthworms. We therefore think that levels of NH_4^+ were not determined by the activity of the measured MB. The experiments with and without earthworms were conducted at the same site and therefore under identical environmental conditions. The external inputs (aeolian and rainfall) were therefore the same. The only remaining possibility the input of NH_4^+ by the mesofauna and particularly by earthworms. This possibility was therefore examined.

Hypothesis II. Increased NH_4^+ production related to earthworm metabolism

Earthworms are known to excrete large quantities of NH_4^+ into the soil as by-products of their metabolism (Parle 1963; Lee 1983). In our experiment, the TIN found in the soil subject to earthworm activity (the low- and the high-elevation sites) was mainly in the form of NH_4^+, which tends to support this second hypothesis (Fig. 3). If the build-up of NH_4^+ was due to earthworm metabolism, there should have been a close correlation between the surplus NH_4^+ and the earthworm biomass. However, when the high- and the low-elevation sites were compared it was apparent that the surplus NH_4^+ found in the two consecutive years when earthworms were present was relatively similar (Fig. 3), but the earthworm biomasses were very different (182 g m^{-2} and 29 g m^{-2} at the low- and the high-elevation sites, respectively). The difference in functional biodiversity between sites is one possible explanation for this result. At the low-elevation site the earthworm population is composed of 93.4% anecics, whereas at the high-elevation site the population is divided almost evenly between anecic (51.7%) and endogeous (44.8%) species. Loquet et al. (1977) showed that, in the field, 42% of aerobic bacteria occurring in the tunnels of anecics consisted of nitrifiers. From this it could be therefore deduced that under aerobic conditions, such as those in the tunnels of anecic species, there was a high rate of nitrification, whereas in the less aerobic conditions existing in the tunnels of endogeous species nitrification

Table 6 Isotopic dilution (MB-E% : NH_4^+-E% ratio) of the three soils at their sites, of origin, +ew and −ew. For abbreviations, see Tables 2 and 3

	Exposure time (days)																							
Soil at site of origin	83			203			300			377			476			595			741			848		
	(+ew)	(−ew)	*t*-test	(+ew)	(−ew)	*t*-test	(+ew)	(−ew)	*t*-test	(+ew)	(−ew)	*t*-test	(+ew)	(−ew)	*t*-test	(+ew)	(−ew)	*t*-test	(+ew)	(−ew)	*t*-test	(+ew)	(−ew)	*t*-test
LES	1.18	1.06	ns	1.34	1.07	*	1.34	1.03	*	1.16	1.06	ns	1.42	1.26	*	1.47	1.19	**	1.38	1.18	ns	1.36	1.21	*
MES	1.21	1.15	ns	1.20	1.16	ns	1.25	1.23	ns	1.36	1.31	ns	1.20	1.26	ns	1.27	1.23	ns	1.38	1.26	ns	1.50	1.47	ns
HES	1.23	1.18	ns	1.39	1.12	**	1.63	1.07	**	1.35	1.07	**	1.32	1.29	ns	1.18	1.06	**	1.50	1.16	*	1.29	1.04	*

$^{**}P < 0.01$; $^{*}P < 0.05$

was slower, leading to an accumulation of NH_4^+. At the low-elevation site, most of the earthworms are anecics, which have tunnels opening to the soil surface; thus it seems reasonable to assume that aerobic conditions prevail and there is a large quantity of nitrifiers. We can therefore put forward the hypothesis, also used by Scheu in a similar study (1987), that a large proportion of the NH_4^+ produced was probably nitrified, despite the acidic soil pH of 5.4. Although nitrification is most efficient at neutral pH it can take place at pH values <6, but becomes negligible at <4.5 (Paul and Clark 1989). The "surplus" NH_4^+ and NO_3^- (Fig. 3) represented the difference between the quantities of NH_4^+ and NO_3^- found in the presence and absence of earthworms. The hypothesis that the total production of NH_4^+ in the presence of earthworms would have been much greater if there was no nitrification in the aerobic tunnels cannot therefore be discounted. Although the surplus NO_3^- found at the low-elevation site was higher than at the high-elevation site (Fig. 3), it did not seem sufficient in order to completely discount this hypothesis. It can be assumed that the majority of the NO_3^- produced was leached or taken up by roots. The herbaceous vegetation was relatively dense at the low-elevation site and the cylinders were invaded by roots, which was not the case at the high-elevation site where this type of vegetation was almost non-existent. At the high-elevation site, despite the presence of anecics (52%), the effect of earthworms on nitrification should have been reduced because of; (1) the lower earthworm biomass, and (2) the unfavourable environmental conditions (cold climate, soil pH). If the accumulation of NH_4^+ was directly related to earthworm metabolism, it was logical that the NH_4^+-E% in the soil should decrease in the presence of earthworms. In fact, when earthworms passed through the experimental cylinders they deposited unlabelled NH_4^+ since they had consumed unlabelled organic matter outside the cylinders. Inside the cylinders, this NH_4^+ from earthworms decreased the level of NH_4^+-E% due to the action of the microflora which decomposed the ^{15}N-labelled humus analogue. At the low-elevation site, in the experimental cylinders, the unlabelled organic matter supplied by earthworms, in the form of litter and faeces, was also used by the in situ microflora. This supply of non-labelled organic matter should have lowered the overall MB-E%; this was not, however, the case, but no convincing explanation could be found for this result.

Our initial objective was to determine the role of climate, soil type and earthworm activity on the decomposition of a humus analogue. Our results can be summed up as follows:

1. Climate and especially soil moisture, played an important role in the decomposition of the humus analogue. Thus, when LES was transplanted to more humid conditions it showed an increase in most of its biological activities (MB-C and MB-N, MB-E%, and NH_4^+-E%). A similar pattern was found in litter-decomposition studies (Cortez 1998; Cortez and Bouché 1998).

2. Under favourable climatic conditions, soil type was a major determinant of the decomposition of the humus analogue. This could have been related to the percentages of clay, silt and sand in soils, that were liable to affect the turnover of the added, labelled, organic matter.

3. The presence of earthworms led to an increase in the quantity of inorganic N available in the soil. This surplus NH_4^+ was more liable to have originated from earthworm metabolism than from MB activity. Furthermore, earthworms stimulated the overall decomposition of native organic matter and the labelled humus analogue to a similar extent. It seemed likely that this stimulation of organic matter decomposition was directly related to earthworm biomass. Hence, the decomposition of the humus analogue and native organic matter was enhanced by the presence of earthworms. In previous experiments we studied the decomposition of leaf litter from four Mediterranean tree species (sessile oak, *Quercus petraea* L.; holm oak, *Quercus ilex* L.; sweet chestnut, *Castanea sativa* and beech, *Fagus sylvatica* L.) in litterbags for more than 2 years in the same sites along the same altitudinal and climatic transect (Cortez 1998; Cortez and Bouché 1998). The effect of earthworms on litter decomposition was negligible during the first year. Thereafter the litter decomposition rate was increased three- to tenfold depending on the litter. This pattern was explained by enhanced litter palatability for earthworms after 1 year in the field, and by litter consumption during the second year. These previous experiments and the present one confirms that the effect of earthworms on organic matter decomposition is mainly linked to organic matter quality. Several authors have reported the importance of organic matter quality on assimilation by earthworms (Martin and Lavelle 1992) and on their growth rate (Cortez and Hameed 1988; Lavelle et al. 1989; Cortez et al. 1993). Cortez and Hameed (1988) also showed a decrease in the growth rate of *Lumbricus terrestris* when the earthworms were fed with lucerne and rye grass for several weeks. They explained this pattern by the loss of easily assimilated organic compounds and the low N availability of the plant material (Abbott and Parker 1981). Finally, our results agree with those of van Rhee (1963), Edwards and Heath (1975), Hartenstein (1982), and Cortez et al. (1989), who showed that there were close relationships between the chemical composition of organic matter and the food preferences of earthworms.

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