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Natural abundance of ^{13}C and ^{15}N in earthworms from different cropping treatments

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Summary

In order to get a better understanding of soil fauna responses to land use changes we used an experimental design established in Devon (UK) and selected five replicated treatments: maize (C_4 plant), grass (C_3 plant), clover (C_3 plant fixing N from the air) and two bicropping systems consisting of maize with a clover understorey. Soil and earthworm samples were taken from each treatment in two sampling occasions (July and October 1998). Results showed that in all treatments the highest C and N contents were measured in the top 10 cm of the soil, with the exception of the maize plots possibly as result of recent rotavation. In contrast soil delta values increased with depth suggesting that agricultural practices affect the distribution of the organic matter through the soil profile. Isotopic composition of the earthworms showed that they were also significantly affected by the cropping systems. In agreement with previous findings N isotope values seem to be related to ecological groupings and ontogenic changes. Furthermore, under bicropping systems endogeic worms seem to gain their nutrition from older residues which is also in correspondence with their vertical distribution in the soil.

Key words: Earthworms, stable isotopes, arable cropping, land use

Introduction

Land use change is expected to be one of the main drivers affecting soil biodiversity and ecosystem functioning (Sala et al. 2000). Earthworms have a strong effect on nutrient dynamics in agricultural soils (Edwards & Bohlen 1996) and knowing their feeding ecology is essential to understand their functional role in these systems. Recently, the use of stable isotope techniques appears to be particularly promising in tracing food relationships in earthworms (reviewed by Scheu 2002).

Overall, these studies conclude that C isotope ratios reflect changes in the diet whereas changes in ^{15}N enrichment allow the description of their trophic status. However, very few attempts have been made in relation to the use of ^{15}N as a tracer of earthworm dietary preferences (e.g. Schmidt et al. 1997). The difference in $\delta^{15}N$ between atmospheric N_2 and plant-available soil N (usually more than 9 delta units) has been widely used as the basis of estimating the relative contribution of atmospheric N_2 to N_2-fixing or-

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ganisms (Shearer & Kohl 1986) and therefore it could be used to investigate food sources transformations in symbiotic systems.

In order to obtain new insights about the feeding relationships of earthworms and to investigate their responses to land use changes we have used stable isotope techniques in a rather more complex way than in previous studies by including several C_3 and C_4 cropping systems (both as monocultures and bicrops) and also in combination with a N_2 – fixing symbioses plant.

Material and Methods

Sampling

We used the experimental design established in Devon on existing C_3 fields and replicated three times (fully described in Jones & Clements 1993). Three monoculture crops: 'conventional maize monocropping' (C_4 plant planted on previous wheat-clover bicropping system), 'grass' (C_3 plant) and 'white clover' (fixing N from the air), and two bricopping systems (maize and clover unserstorey): 'bicropping-old' (established on existing 3 years clover sward) and 'bicropping-new' (never had clover or maize mono cultures in the past) were selected.

Sampling was conducted in 1998 in two sampling occasions (at the maximum of the maize growth, in July, and one month after the maize was harvested, in October). Harvesting of the maize involved the removal of the plants from the field but leaving the roots in the soil.

Soil and earthworm samples were randomly taken from each replicated plot and treatment. Three soil samples from three different depths (0–10, 10–20 and 20–30 cm) from each plot and treatment were taken on every sampling occasion and were oven-dried to 65 °C to constant weight. The worms were obtained by careful hand-sorting in the field, then taken to the lab, killed by dipping for 1 s into boiling water, dissected to remove the guts, and rapidly frozen to -10 °C until further analysis. When possible, three individuals from each earthworm species per cropping treatment were dissected at each sampling date and their ecological (epigeic, anecic and endogeic) and age grouping (mature = clitellum developed, semi-mature = only tubercula present, immature = absence of sexual characteristics) annotated.

Isotopic analysis

All samples (replicate animal and soil samples) were prepared and analysed following previous methodology (fully described in Briones et al. 1999a).

Statistical analysis

Comparisons between treatments, sampling dates, soil depths, earthworm age groups and their ecological categories used analysis of variance (ANOVA). One-way ANOVA was used to compare: (i) mean soil C and N content and isotopic values per treatment, soil depth and sampling date, (ii) mean isotope values of the earthworm functional and age groups per treatment and sampling date and (iii) mass balance calculations of tissue delta values from the two bicropping systems. Separation of means was determined using Tukey's Studentized Range (HSD) test ($\alpha = 0.05$).

Two-way ANOVA was used to quantify interactions between sampling dates, treatments, soil depths and earthworms' ecological and age groupings.

Results

Soils

Date and depth had a significant effect on carbon and nitrogen content and delta values of the soils. A sig-

Table 1. Results from the Analysis of Variance (ANOVA) for % C, % N, $\delta^{13}C$ and $\delta^{15}N$ values of the soils

	DF	% C		% N		$\delta^{13}C$		$\delta^{15}N$	
		F	P > F	F	P > F	F	P > F	F	P > F
DATE	1	16.00	0.0002	44.39	0.0001	49.89	0.0001	84.70	0.0001
TREAT	4	1.80	0.1395	4.50	0.0027	0.84	0.5038	9.08	0.0001
DEPTH	2	10.16	0.0001	10.72	0.0001	3.16	0.0488	9.23	0.0003
TRAET*DATE	4	1.14	0.3453	1.53	0.2038	3.01	0.0241	3.20	0.0180
TREAT*DEPTH	8	0.92	0.5058	1.08	0.3871	1.76	0.1016	1.33	0.2462
DEPTH*DATE	2	0.97	0.3849	0.63	0.5364	0.34	0.7141	1.37	0.2604

DATE = Sampling dates, TREAT = plots of maize, grass, clover, bi-cropping old and bi-cropping new, DEPTH = 0–10 cm, 10–20 cm, 20–30cm

Table 2. Results from the Analysis of Variance (ANOVA) for % C, % N, $\delta^{13}C$ and $\delta^{15}N$ values of the soils at the three depths from each treatment. Values are means with standard errors

	GRASS			MAIZE			CLOVER			BI-OLD			BI-NEW		
%C															
0–10 cm	1.78	(0.21)	**a**	1.80	(0.25)	**a**	2.06	(0.42)	**a**	2.00	(0.37)	**a**	1.82	(0.24)	**a**
10–20 cm	1.69	(0.36)	**a**	1.89	(0.29)	**a**	1.71	(0.21)	**ab**	1.66	(0.35)	**a**	1.53	(0.17)	**ab**
20–30 cm	1.62	(0.37)	**a**	1.48	(0.41)	**a**	1.63	(0.31)	**b**	1.68	(0.55)	**a**	1.33	(0.10)	**b**
%N															
0–10 cm	0.20	(0.02)	**a**	0.21	(0.03)	**a**	0.23	(0.04)	**a**	0.22	(0.03)	**a**	0.19	(0.02)	**a**
10–20 cm	0.19	(0.04)	**a**	0.22	(0.03)	**a**	0.20	(0.02)	**ab**	0.19	(0.03)	**a**	0.17	(0.02)	**ab**
20–30 cm	0.18	(0.04)	**a**	0.18	(0.04)	**a**	0.19	(0.03)	**b**	0.19	(0.05)	**a**	0.16	(0.01)	**b**
$\delta^{13}C$															
0–10 cm	-29.08	(0.30)	**a**	-28.97	(0.45)	**a**	-28.88	(0.51)	**a**	-28.96	(0.25)	**a**	-28.96	(0.39)	**a**
10–20 cm	-28.71	(0.35)	**b**	-28.64	(0.39)	**a**	-28.70	(0.37)	**a**	-29.07	(0.26)	**a**	-28.86	(0.14)	**a**
20–30 cm	-28.77	(0.28)	**b**	-29.09	(0.49)	**a**	-28.83	(0.34)	**a**	-28.83	(0.29)	**a**	-28.91	(0.24)	**a**
$\delta^{15}N$															
0–10 cm	5.19	(0.60)	**a**	5.57	(0.56)	**a**	4.97	(0.76)	**a**	5.47	(0.63)	**a**	5.60	(0.53)	**a**
10–20 cm	5.53	(0.97)	**ab**	5.16	(0.59)	**a**	5.30	(0.58)	**a**	6.14	(0.77)	**a**	5.95	(0.56)	**ab**
20–30 cm	5.88	(0.79)	**b**	5.57	(0.70)	**a**	5.32	(0.37)	**a**	6.42	(1.47)	**a**	6.36	(0.60)	**b**

Table 3. Results from the Analysis of Variance (ANOVA) for $\delta^{13}C$ and $\delta^{15}N$ values of the earthworm tissues

	DF	$\delta^{13}C$		$\delta^{15}N$	
		F	P>F	F	P>F
TREAT	4	10.11	0.0001	18.00	0.0001
DATE	1	0.96	0.3289	5.07	0.0263
ECOL	2	130.19	0.0001	86.00	0.0001
AGE	2	13.44	0.0001	7.72	0.0007
TREAT*DATE	4	1.87	0.1215	1.98	0.1029
TREAT*ECOL	6	0.00	1.0000	5.68	0.0001
TREAT*AGE	7	0.00	1.0000	6.03	0.0001
DATE*ECOL	2	3.79	0.0257	5.69	0.0045
DATE*AGE	2	12.08	0.0001	11.34	0.0001
ECOL*AGE	3	2.67	0.0513	0.54	0.6547

Table 4. Results from the Analysis of Variance (ANOVA) for C and N content in the earthworm tissues derived from a specific source in the two bicropping systems

	DF	C derived from maize (mg g worm^{-1})		N derived from clover (mg g worm^{-1})	
		F	P>F	F	P>F
TREAT	1	4.54	0.0407	40.60	0.0001
DATE	1	1.96	0.1705	11.39	0.0019
SPECIES	4	3.68	0.0138	17.73	0.0001
ECOL	1	12.37	0.0013	51.82	0.0001
TREAT*DATE	1	0.00	1.0000	3.85	0.0581
TREAT*SPECIES	3	9.44	0.0001	8.97	0.0002
TREAT*ECOL	1	27.37	0.0001	24.25	0.0001
DATE*SPECIES	2	2.95	0.0663	0.00	1.0000

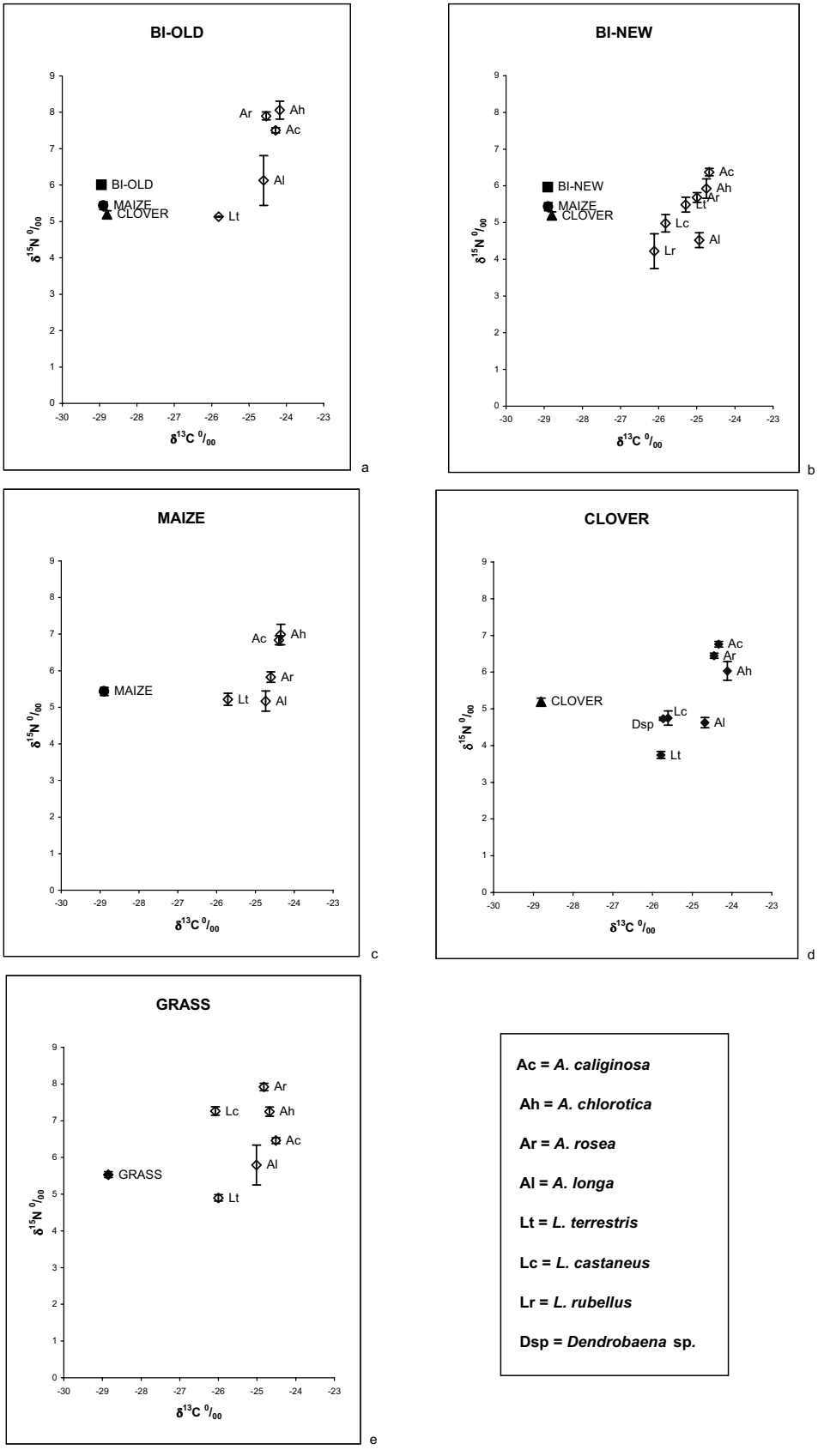

Fig. 1. Plots showing $\delta^{13}C$ and $\delta^{15}N$ values for the earthworm species in the five treatments. 1. Species abbreviations: *Allolobophora caliginosa* (Ac), *A. rosea* (Ar), *A. chlorotica* (Ah), *A. longa* (Al), *Lumbricus terrestris* (Lt), *L. rubellus* (Lr), *L. castaneus* (Lc), *Dendrobaena sp.* (Dsp)

nificant treatment effect was observed for nitrogen content and ^{15}N delta values. Significant interactions were only obtained between date and treatment for isotope values (Table 1).

In all treatments the highest C and N contents were measured in the top 10 cm of the soil, with the exception of the maize plots possibly as result of rotavation (Table 2). A significant increase in $\delta^{13}C$ values with depth ($P < 0.05$) was observed in the soils under grass. ^{15}N delta values also increased with depth with the exception of the maize plots, but significant differences were only detected for the grass and bi-new treatments.

Earthworms

Eight earthworm species belonging to different ecological categories were analysed: three endogeic worms (*Allolobophora caliginosa*, *A. chlorotica*, *A. rosea*), two anecic (*A. longa*, *Lumbricus terrestris*) and three epigeic (*L. rubellus*, *L. castaneus*, and immatures of *Dendrobaena* sp.)

Treatment, ecological group and age groupings had a significant effect on the isotopic composition of the earthworms (Table 3). Significant interactions between sampling dates and age and ecological groupings were also obtained for delta values of the earthworm tissues, and between treatments and ecological and age groupings for $\delta^{15}N$ values only.

Figure 1 shows the plots of $\delta^{13}C$ versus $\delta^{15}N$ values of the earthworms and soils in the different treatments under study. Soil values in all treatments showed strong ^{13}C depletion (3 delta units) when compared to those of the tissue ($P < 0.05$). Earthworm $\delta^{15}N$ values showed a variable range between 3.7‰ and 8‰; in contrast, soil values showed a

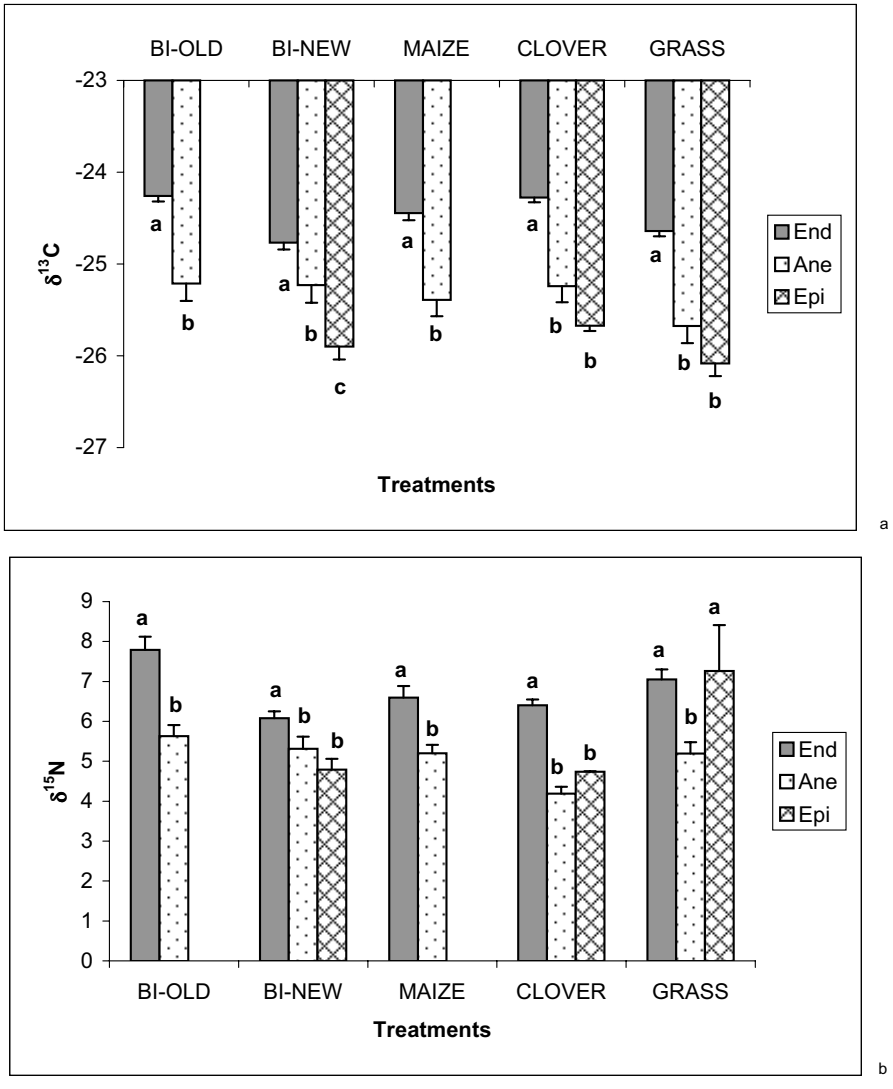

Fig. 2. $\delta^{13}C$ (a) and $\delta^{15}N$ (b) values of the different ecological categories of the earthworms in each treatment and results from one-way ANOVA with different letters indicating significant differences. Units are ‰

small difference, less than one delta unit between the different treatments.

When focussing on the ecological groupings (Fig. 2a), endogeic worms were significantly different from anecics and epigeics in all treatments ($P < 0.05$) showing less negative values in their C isotopic composition. Ecological groups also showed different responses in relation to $\delta^{15}N$ values (Fig. 2b), in particular endogeics showed significantly higher N isotope values than the other groups in all treatments except in the grassland where the epigeic worm *L. castaneus* showed a similar feeding behaviour to the endogeics. Mean $\delta^{15}N$ values of all groups were at least 1 delta unit lower in the clover than in the rest of the treatments, suggesting that in the bicrops their N is not derived mainly from these residues.

To determine where the worms collected under the two bicropping systems were obtaining their C and N sources, mass balance calculations of the delta values were obtained. Treatment, individual species and their trophic position had a significant effect on the values (Table 4). And thus, endogeic species (*A. caliginosa* and *A. chlorotica*) collected under "bicropping old" contained more C from maize and more N from clover (as mg g worm^{-1}) than under "bicropping new" ($P < 0.05$). The anecic worm *L. terrestris* did not show any significant difference in its C and N content between the two bicropping systems, but individuals under "bicropping new" contained significantly more C and N from source than the two endogeic species in the same treatment ($P < 0.05$).

Due to the impossibility of capturing worms of different age for each species and sampling date, age groups were established by pooling the values to give just the means per treatment (Fig. 3). Significant differences for ^{13}C values were only detected for bi-old where mature and semi-mature worms were more enriched than immature ones (Fig. 3a). N isotope ratios showed that mature worms tend to feed on older material than the immature ones but significant differences were only observed for clover and grass (Fig. 3b).

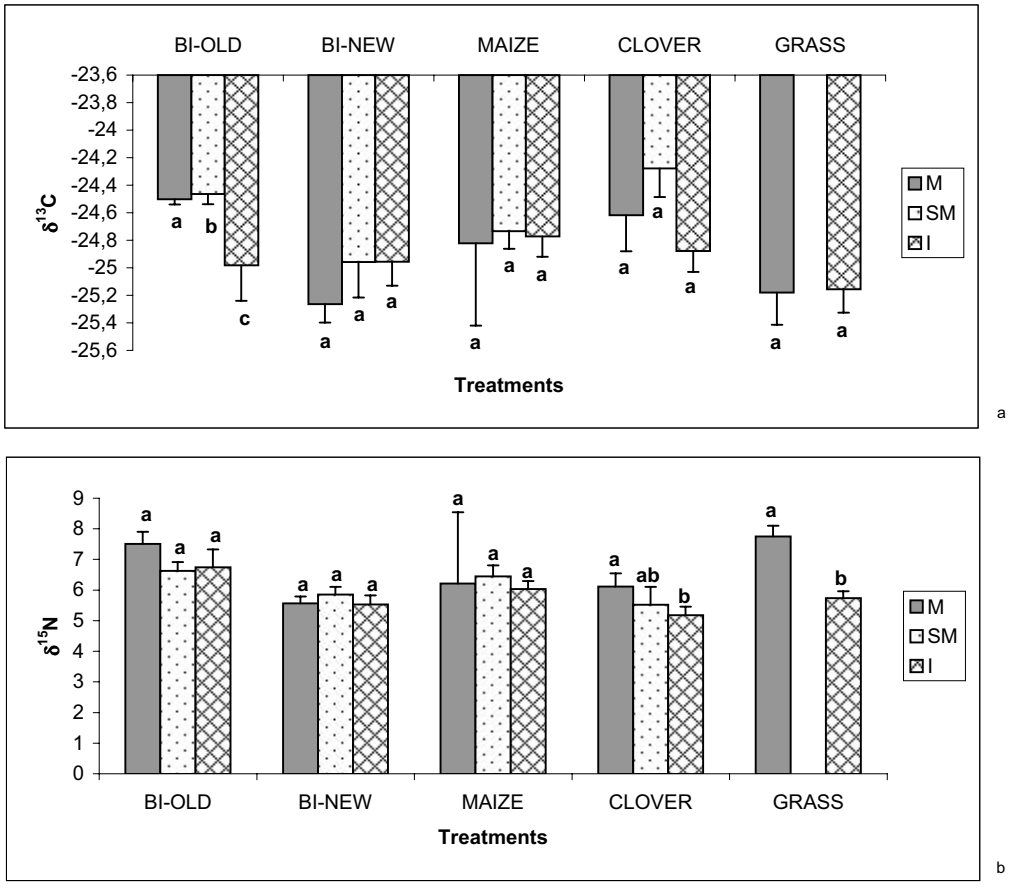

Fig. 3. $\delta^{13}C$ (a) and $\delta^{15}N$ (b) values of the different earthworm age groups in each treatment and results from one-way ANOVA with different letters indicating significant differences. Units are ‰

Discussion

C isotope values increase with soil depth as a result of decomposition processes (e.g. Handley & Scrimgeour 1997) and in our cropping systems this increase was only significant for the grassland soils. This is possibly due to the agricultural practices and rotavation prior to the establishment of the different crops.

In contrast, the increase of $\delta^{15}N$ values with depth is usually related to a more intense microbial activity (e.g. Balesdent et al. 1993) and therefore earthworms feeding in deeper profiles would have access to more mineralised organic matter. Interestingly, soils under clover did not appear to be strongly depleted in ^{15}N as it is expected to be from a N_2 – fixing symbiotic plant. This suggests that the observed delta ^{15}N values are reflecting those from the past vegetation (non-fixing), which gets N from soil N pool which is enriched compared to the atmospheric value. Although clover has been the main crop over the last five years, this was not enough time to replace all soil N with clover derived N (isotopically distinct).

In terms of earthworm feeding preferences, isotopic data did not allow the differentiation of C_3 versus C_4 plants. This is possibly due to either the amount of time required for the C_4 material to be incorporated into the soil (and hence to be available to the worms), or to agricultural practices (including crop rotations) which could have obscured the tracer signal. However, delta values did provide valuable information in determining from which source the earthworms derive the C and N present in their tissues. And, in relation to this, our results showed that *A. caliginosa* and *A. chlorotica* seem to assimilate C and N derived from older residues than *L. terrestris* which also fits with their ecological grouping position.

Although it is difficult to delineate trophic levels for such detritivores as earthworms (Scheu 2002), several authors have related N isotope values to their feeding ecological groups (Schmidt et al. 1997; Hendrix et al. 1999a,b; Briones et al. 1999a,b; 2001) leading to the conclusion that endogeics are more ^{15}N enriched than epigeics due to their preferential feeding on more microbially processed organic matter. Accordingly, anecics should then fall in the middle of this classification as a result of their vertical movements through the soil profile. However, our data showed that the anecic group formed here, by *L. terrestris* and *A. longa* did not significantly differ from the epigeics with the exception of the undisturbed grassland. Indeed *L. terrestris* has been reported to have feeding attributes similar to either epigeic (Jégou et al. 1999), to endogeic (Bernier 1998) or to both (epi/anecic) depending of the seasonal availability of the litter (Bouché 1971). These isotopic variations have led some authors to question this classification arguing that the observed differences are indicative of habitat diversity (Edwards & Bohlen 1996; Neilson et al. 2000).

In both natural and agroecosystems soil biota are responsible for performing vital functions in the soil ecosystem and the analysis of the variation of stable isotopes at natural levels can provide an estimate of land use changes on ecosystem structure and function.

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