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Spatio-temporal variation of stable isotope ratios in earthworms under grassland and maize cropping systems

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Abstract

We investigated the specific diet and habitat of earthworms in relation to land use changes by integrating spatial and temporal scales and by using stable isotope (^{13}C and ^{15}N) techniques. The study involved two sites: Santiago (Northwest Spain) and North Wyke (Southwest England), both consisting of long term grassland which was partly converted to a maize crop in 1997. In 1998, the maize crop in Spain was divided into two, and one half was re-planted with maize (2 years maize) and the other half reconverted to grassland (1 year grassland); the same procedure was followed for the grassland resulting in two treatments, 2 years grassland and 1 year maize. At the English site only 2 years maize and 2 years grassland were under investigation. Within each of the four treatments in Spain and the two in England, three replicate plots were established.

Random soil samples from three different depths (0–10, 10–20 and 20–30 cm), and earthworm specimens belonging to four different ecological categories (epigeic, anecic, epi/anecic and endogeic), were taken from each plot, treatment and site at the peak of the maize growth and after harvesting.

Spanish soils were ^{13}C-enriched and ^{15}N-depleted when compared to the English ones, which was also reflected in the earthworm tissue, and allowed a direct relationship between the delta values of the animals and the cropping treatments.

The enrichment in the ^{13}C values of the worms feeding on the maize (C_4) plots, when compared to those found under the grassland (C_3) plots, was greater than the difference detected in the C_4 vs C_3 soils. This result clearly indicates selective feeding by earthworms with a preference for fresh C_4 residue over older native C_3.

Different ecological and age groups appeared to consume organic material of differing quality, with endogeic species and mature worms showing the highest N isotope values as a result of preferential feeding in deeper soil profiles. This information proves that combined C and N isotope analysis constitutes a powerful tool in studying feeding ecology and emphasises the need for long-term studies which incorporate spatial and temporal scales to the experimental set-up.

Keywords: Earthworms; Stable isotopes; Carbon; Nitrogen; Diet

1. Introduction

Human activities and climate change are expected to alter the agricultural landscape and warmer conditions, as anticipated under many global warming scenarios, will permit the conversion of large areas of temperate grasslands (C_3) to arable crops, such as maize (C_4). These changes in land use can have a substantial influence upon the direction and magnitude of the responses to global change of individual, population, community and whole soil ecosystem.

Soil fauna plays an important role in nutrient dynamics, and earthworms in particular have a strong effect on both soil properties and biogeochemical cycles through their interactions with microbial populations. It is generally accepted that earthworm species can be classified into ecological categories according to their feeding behaviour and burrowing activities as well as other morphological and physiological characteristics (Bouché, 1972; Lee, 1985; Lavelle et al., 1993). These dietary preferences have been investigated by using palatability tests, analysing their gut contents and more recently applying tracer techniques (Schmidt et al., 1997). However, it is unlikely that this functional classification can be successfully applied to all earthworm species and therefore new information is needed

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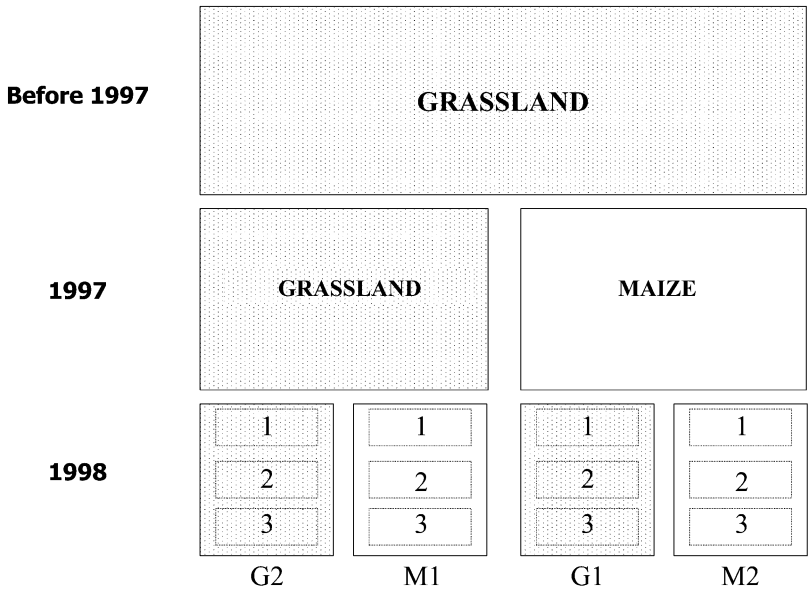

Fig. 1. Experimental design showing the treatments established and the layout of the plots during the 2 years experiment at both sites. Abbreviations: M1 = maize 1 year, M2 = maize 2 years, G1 = grassland 1 year, G2 = grassland 2 years.

to obtain quantitative estimates of dietary preferences and energy fluxes between the different soil compartments.

Isotopic techniques have proved to be a powerful tool in soil ecology studies by providing valuable information about flux rates between organisms and their environment at population and ecosystem levels. Although isotopic studies can vary in their application most studies have explored the possibility of differentiating natural variations in isotopic values of the substrate sources (marine vs terrestrial origin, C_3 vs C_4) (Owens, 1987; Peterson and Fry, 1987; Gearing, 1991; Gannes et al., 1998; Hobson, 1999; Fantle et al., 1999). Soil ecology has greatly benefited from these tools by gaining new insights into our understanding of the structure and function of the soil ecosystem. Since soil organic matter is an extremely heterogeneous environment, it is difficult for soil ecologists to identify and distinguish between the complex food sources ingested and assimilated by soil biota. Therefore, natural abundances of stable isotopes have been successfully applied to determine feeding preferences and resource partitioning in a number of invertebrates, mainly earthworms (Martin et al., 1992a,b; Schmidt et al., 1997, 1999; Hendrix et al., 1999a,b; Briones et al., 1999a,b). From these studies it has been concluded that the combined use of C and N ratios offers more valuable information than single isotope studies (Spain and Le Feuvre, 1997; Neilson and Brown, 1999). This is of special interest when diets have overlap ranges of $\delta^{13}C$ values, and has proved to be of great potential in ecophysiological studies on internal nutrient allocation and elemental turnover (Schmidt et al., 1999).

It is also necessary to integrate both temporal and spatial scales to investigate how not only individual populations but also the whole ecosystem respond to the different variables responsible for global change. Thus, if due to resource changes (e.g. as result of land use change) different species move through the soil profile and co-exist in the same habitat, increased competition might force some species to change to a different diet. This could lead to changes in resource partitioning in the community structure of soil fauna, with important effects on biogeochemical cycles (Swift and Anderson, 1993; Swift et al., 1998).

In order to achieve this, chronosequence studies could provide a better understanding of the soil system and, therefore, in the work reported here we investigated the specific diet and habitat of earthworms in relation to land use changes by integrating spatial and temporal scales and by using stable isotope (^{13}C and ^{15}N) techniques.

2. Material and methods

2.1. Sampling

The study involved two sites: Neiro, Santiago de Compostela, Spain (42°53′N, 8°27′W) and North Wyke, Okehampton, Devon, England (50°48′N, 4°38′W). At both sites a long term grassland was partly converted to a maize crop in 1997 (Fig. 1). In 1998 the maize crop in Spain was divided into two parts and one half was re-planted with maize (2 years maize, M2) and the other half reconverted to grassland (1 year grassland, G1); the same procedure was followed for the grassland resulting in two treatments, 2 years grassland (G2) and 1 year maize (M1) (Fig. 1). At the English site only 2 years maize and 2 years grassland were under investigation.

Within each of the four treatments in Spain (G1, G2, M1, M2) and the two in England (G2, M2), three replicate plots were established and random soil and earthworm samples

were taken. Sampling was performed at the maximum of the maize growth (25 August 1998) in Spain, and on three occasions after the maize was harvested at both sites (Spain: 20 October 1998, 4 December 1998 and 5 March 1999; England: 16 October 1998, 3 December 1998 and 10 March 1999).

Earthworms samples 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. The worms to be dissected were selectively chosen in order to get a representation of the different ecological categories: epigeic, epi/anecic, anecic and endogeic. Thus, in Spain two endogeic (*Allolobophora caliginosa*, *A. rosea*), two epigeic (*Dendrobaena octaedra*, *D. rubida*), one epi/anecic (*Lumbricus festivus*) and one anecic (*A. trapezoides*) earthworm species were collected, while in England three endogeic (*A. caliginosa*, *A. rosea*, *A. chlorotica*), three epigeic (*L. rubellus*, *L. castaneus* and *D. mammalis*) and one anecic (*A. longa*) were collected. Three specimens of each species were individually prepared for isotopic analysis. When possible, three mature individuals of each earthworm species were selected, but semi-mature (with tubercula pubertatis) and immature worms were also considered when no mature worms were available.

Three soil samples from three different depths (0–10, 10–20 and 20–30 cm) were taken from each plot within each treatment and these were pooled together to give one sample per depth and plot in Spain and two in England (Spain: $N = 1$ replicate × 3 soil depths × 3 plots × 4 treatments = 36, England: $N = 2$ replicates × 3 depths × 3 plots × 2 treatments = 36). These random replicate soil samples from each depth at each plot, treatment and site were taken on every sampling occasion and were oven-dried to 65°C to a constant weight for 7 days.

2.2. Isotopic analysis

Every single earthworm specimen of each species, from every treatment, sampling date and site was freeze-dried for N and C isotopic analysis. All samples (replicate animal and soil samples) were ground to a homogeneous powder using 'SPEX' liquid nitrogen cooled mill (Glen Creston Ltd., Stanmore, Middlesex, UK) before being weighed using a micro-balance to ensure sufficient dry-weight for each analysis. Earthworm samples were sealed into 6 × 4 mm tin capsules prior to separate C and N isotope analyses at Merlewood, using an ANCA-MS system (Europa Scientific Ltd, Crewe, Cheshire, UK); this analytical system is fully described by Barrie and Prosser (1996). Beet sucrose was used as a reference material for C, at −25.96‰, relative to PDB. Ammonium sulphate, at +1.74‰ relative to air (atmospheric N), was employed for N. Analytical precision over 10 replicates of both standards was better than 0.2‰. Soil samples were similarly analysed at Aberystwyth against a Europa flour standard $\delta^{13}C = -25.34‰$ and $\delta^{15}N = 3.01‰$.

2.3. Statistical analysis

Comparisons between sites, treatments, sampling dates, soil depths, earthworm age groups and their ecological categories were done using analysis of variance (ANOVA). One-way ANOVA was used to compare mean soil C and N content and isotopic values per site, treatment, soil depth and sampling date, and to compare mean isotope values of the earthworm functional and age groups per site, treatment and sampling date. Separation of means was determined by using Tukey's Studentized Range (HSD) test ($\alpha = 0.05$).

Two-way ANOVA was used to test for significant differences in soil C and N content and isotopic values between sites by treatments, by sampling dates and soil depths, among treatments by dates and soil depths and among soil depths by sampling dates. A similar two-way ANOVA was used to test for significant differences in earthworm isotopic values between sites by treatments, sampling dates and ecological and age groupings, among treatments by dates, ecological groups and age, among dates by ecological groups and age, and among ecological groups by age.

3. Results

3.1. Soils

Site, treatment, date and depth had a significant effect on carbon and nitrogen content and the delta values of soils. Significant interactions were only obtained for $\delta^{13}C$ values and $\delta^{15}N$ (Table 1).

Independently of the treatment, sampling date and soil depth, average values of Spanish soils showed that they contained significantly ($P < 0.05$) more carbon and nitrogen than the English ones, three times more carbon and twice the amount of nitrogen. At the Spanish soils $\delta^{13}C$ values were, on average, 1.8‰ greater than at the English site ($P < 0.05$) whereas $\delta^{15}N$ values of the English soils were, on average, 1.9‰ greater than the Spanish ones ($P < 0.05$).

When comparing treatments at each site, the average values of the pasture plots at the Spanish site were significantly ($P < 0.05$) higher in C content than those under maize cropping (values decreased in the order G2 ≥ G1 > M2 > M1), and no significant differences were found at the English site. Higher N contents were measured in the grassland plots at both sites, but significant differences ($P < 0.05$) were only detected at the English site (G2 > M2) and between M1 and G2 treatments at the Spanish site.

Table 2 shows the mean C and N contents of the soil samples at each depth per treatment and site. It can be seen that in all treatments the highest C and N values were generally measured in the upper 10 cm of the soil, with the exception of the maize plots at the Spanish site probably as result of rotavation.

A significant decrease in ^{13}C values with depth (Table 3) was observed in the English plots ($P < 0.05$), whereas at the

Table 1
Results from the Analysis of Variance (ANOVA) for %C, %N, $\delta^{13}C$ and $\delta^{15}N$ values of the soils (SITE, Spain, England; DATE, sampling dates; TREAT, plots of maize and grassland (M1, M2, G1, G2); DEPTH, 0–10, 10–20, 20–30 cm)

	DF	%C		%N		$\delta^{13}C$		$\delta^{15}N$	
		F	*P* > *F*	*F*	*P* > *F*	*F*	*P* > *F*	*F*	*P* > *F*
SITE	1	571.03	0.0001	564.74	0.0001	452.56	0.0001	501.02	0.0001
DATE	6	95.56	0.0001	94.86	0.0001	76.35	0.0001	96.34	0.0001
TREAT	3	56.91	0.0001	49.87	0.0001	43.71	0.0001	41.13	0.0001
DEPTH	2	19.92	0.0001	24.41	0.0001	9.68	0.0001	26.35	0.0001
SITE*TREAT	1	0.00	1.0000	0.00	1.0000	0.00	1.0000	0.00	1.0000
SITE*DATE	0	–	–	–	–	–	–	–	–
SITE*DEPTH	2	0.36	0.6984	1.48	0.2313	3.86	0.0227	6.04	0.0029
TREAT*DATE	12	0.00	1.0000	0.00	1.0000	0.00	1.0000	0.00	1.0000
TREAT*DEPTH	6	0.71	0.6421	1.35	0.2369	1.62	0.1424	2.26	0.0396
DEPTH*DATE	12	0.63	0.8129	0.82	0.6340	1.71	0.0678	2.49	0.0048

Table 2
Results from the Analysis of Variance (ANOVA) for %C and %N soil contents at the three depths from each treatment at both sites. Values are means with standard errors. Abbreviations as in Fig. 1

	Spain				England	
	M1	M2	G1	G2	M2	G2
%C						
0–10 cm	7.33 (0.35)**a**	7.34 (0.31)**a**	9.13 (0.25)**a**	9.91 (0.33)**a**	3.73 (0.13)**a**	4.05 (0.25)**a**
10–20 cm	7.20 (0.46)**a**	7.76 (0.31)**a**	8.52 (0.20)**ab**	8.25 (0.24)**b**	2.78 (0.19)**b**	2.42 (0.09)**b**
20–30 cm	6.36 (0.26)**a**	7.83 (0.46)**a**	8.03 (0.28)**b**	8.07 (0.30)**b**	1.55 (0.13)**c**	1.48 (0.10)**c**
%N						
0–10 cm	0.71 (0.03)**ab**	0.72 (0.03)**a**	0.78 (0.02)**a**	0.83 (0.01)**a**	0.40 (0.01)**a**	0.48 (0.02)**a**
10–20 cm	0.75 (0.03)**a**	0.75 (0.02)**a**	0.75 (0.01)**a**	0.70 (0.02)**b**	0.33 (0.01)**b**	0.34 (0.01)**b**
20–30 cm	0.62 (0.02)**b**	0.69 (0.02)**a**	0.65 (0.02)**b**	0.73 (0.02)**b**	0.22 (0.02)**c**	0.25 (0.02)**c**

Table 3
Results from the Analysis of Variance (ANOVA) for $\delta^{13}C$ and $\delta^{15}N$ soil values at the three depths from each treatment at both sites. Values are means with standard errors. Abbreviations as in Fig. 1

	Spain				England	
	M1	M2	G1	G2	M2	G2
$\delta^{13}C$						
0–10 cm	−25.67 (0.11)**a**	−25.56 (0.10)**a**	−25.83 (0.07)**a**	−25.81 (0.06)**a**	−27.80 (0.10)**a**	−27.64 (0.19)**a**
10–20 cm	−25.69 (0.08)**a**	−25.52 (0.10)**a**	−25.72 (0.08)**ab**	−25.63 (0.10)**a**	−27.84 (0.09)**a**	−27.18 (0.19)**a**
20–30 cm	−25.63 (0.11)**a**	−25.52 (0.08)**a**	−25.59 (0.07)**b**	−25.62 (0.05)**a**	−27.50 (0.09)**b**	−26.41 (0.29)**b**
$\delta^{15}N$						
0–10 cm	5.62 (0.27)**a**	5.45 (0.16)**a**	5.20 (0.15)**a**	4.92 (0.23)**a**	6.80 (0.11)**a**	6.61 (0.20)**a**
10–20 cm	5.47 (0.28)**a**	5.26 (0.19)**a**	5.33 (0.14)**a**	5.73 (0.17)**b**	7.32 (0.10)**b**	7.58 (0.12)**b**
20–30 cm	5.80 (0.18)**a**	5.59 (0.19)**a**	5.62 (0.16)**b**	5.49 (0.18)**b**	7.76 (0.13)**c**	8.10 (0.15)**c**

Spanish site only the 1 year grassland treatment showed a similar trend. In contrast, $\delta^{15}N$ values generally increased with soil depth at both sites (Table 3). At the Spanish plots this trend was more pronounced in the grassland than in the arable plots, as a result of rotavating the soil prior to establishment of the maize.

Additionally, soil $\delta^{13}C$ values (0–10 cm depth) in the grassland plots at the English site and in all plots at the Spanish site increased from winter to early spring but differences were not significant. Only at the Spanish site was a seasonal trend in the $\delta^{15}N$ values (0–10 cm depth) of the grassland soil observed. There was a significant decrease from late summer to late autumn ($P < 0.05$), followed by a slight increase in winter to final minimum values (at 0–10 and 10–20 cm depths) in early spring ($P < 0.05$) (Table 4).

3.2. Earthworms

Site, treatment, date and ecological and age groupings

Table 4
Results from the Analysis of Variance (ANOVA) for $\delta^{15}N$ soil values in the grassland plots (G1 = grassland 1 year; G2 = grassland 2 years) at the Spanish site with different letters showing significant differences between dates at each soil depth. Values are means with standard errors

	G1				G2			
	August	October	December	March	August	October	December	March
0–10 cm	5.8633 (0.1181)**a**	4.7848 (0.1886)**bc**	5.4044 (0.0974)**ab**	4.7451 (0.1322)**c**	5.5453 (0.3051)**a**	4.5524 (0.1488)**b**	5.6241 (0.2149)**a**	3.9576 (0.0042)**b**
10–20 cm	5.8729 (0.1240)**a**	5.0435 (0.0705)**b**	5.5017 (0.0953)**a**	4.6915 (0.0124)**b**	5.8690 (0.2812)**a**	5.8793 (0.1047)**a**	6.1682 (0.2291)**a**	5.0354 (0.2841)**b**
20–30 cm	6.5329 (0.2790)**a**	5.3059 (0.1404)**b**	5.6188 (0.0586)**b**	5.3412 (0.2027)**b**	5.8456 (0.1251)**ab**	4.7656 (0.1875)**b**	6.0100 (0.2035)**a**	5.3413 (0.3766)**ab**

had a significant effect on delta values but the interaction between these factors was only significant for $\delta^{13}C$ values (Table 5).

The differences observed in the isotope values of the soils from the two sites were also reflected in the earthworm tissues and, therefore, higher $\delta^{13}C$ and lower $\delta^{15}N$ values were measured in the Spanish earthworm tissues when compared to the English ones.

Treatment had an effect on the isotopic composition of the animals and this was particularly clear for C at both sites, where the earthworms collected in the maize plots showed higher isotopic values than those under grassland [Fig. 2(a)]. Furthermore, at the Spanish site the difference between the $\delta^{13}C$ values of the two maize treatments was also significant ($P < 0.05$). Similarly, higher N isotope values were measured in the worms feeding under maize, especially at the English site [Fig. 2(b)].

At both sites, stable C isotope ratios in the earthworm tissues reflected preferential assimilation of C derived from the maize with $\delta^{13}C$ values becoming less negative with time, and it was possible in all cases to distinguish between specimens belonging to the same species but under different treatments (Fig. 3). This temporal trend was particularly clear for *A. caliginosa* and *A. rosea* at the Spanish site which showed the highest C isotope ratios at the end of the sampling period. At the English site although the earthworms did not reach such high delta values during the course of the experiment it was possible to identify their food source.

Table 5
Results from the Analysis of Variance (ANOVA) for $\delta^{13}C$ and $\delta^{15}N$ values of the earthworm tissues (SITE, Spain, England; DATE, sampling dates; TREAT, maize and grassland plots (M1, M2. G1, G2); ECOL, epigeic, epi/anecic, anecic, endogeic; AGE, mature, semi-mature, immature)

	DF	$\delta^{13}C$		$\delta^{15}N$	
		F	*P* > *F*	*F*	*P* > *F*
SITE	1	577.70	0.0001	174.65	0.0001
DATE	6	111.41	0.0001	29.38	0.0001
TREAT	3	274.52	0.0001	23.20	0.0001
ECOL	3	152.51	0.0001	81.40	0.0001
AGE	2	3.27	0.0407	19.59	0.0001
SITE*DATE	0	–	–	–	–
SITE*TREAT	1	0.00	1.0000	0.00	1.0000
SITE*ECOL	2	47.23	0.0001	0.00	1.0000
SITE*AGE	2	40.51	0.0001	0.00	1.0000
DATE*TREAT	12	4.11	0.0001	0.00	1.0000
DATE*ECOL	12	15.68	0.0001	0.00	1.0000
DATE*AGE	12	21.91	0.0001	1.63	0.0888
TREAT*ECOL	9	4.62	0.0001	0.00	1.0000
TREAT*AGE	6	40.14	0.0001	0.35	0.9087
ECOL*AGE	6	13.85	0.0001	0.00	1.0000

The ecological grouping had a significant effect ($P < 0.05$) on $\delta^{13}C$ values with endogeic species being significantly different from epigeic species at both sites [Fig. 4(a) and (b)] and to epi/anecic species at the Spanish site. Treatment also had an effect on the ecological groups of earthworms. Thus, all groups in the Spanish soils, with the exception of epigeic species, showed significant differences between maize and grassland treatments after 2 years. Remarkably, the epi/anecic worm *L. festivus* showed the greatest difference in isotopic values (ca. two delta units) between the 2 years maize and 1 year grassland treatments. The epigeic species in the English maize plots were two delta units enriched compared to the ones found under grassland ($P < 0.05$). Endogeic worms, however, seemed to be the least responsive, i.e. less than one delta unit difference between the two treatments, whereas the difference obtained for anecic worms, although slightly higher than one delta unit, was not statistically significant.

^{15}N values also differed between ecological groups at both sites [Fig. 4(c) and (d)] with endogeic species being significantly different from the other groups ($P < 0.05$) and the epi/anecic worm *L. festivus* at the Spanish site showing the lowest values. However, no clear relationship between ecological groupings and the cropping treatments was observed and only endogeic species at both sites and epi/anecic species at the Spanish site seemed to be significantly affected by treatments.

Unfortunately, it was not always possible to capture worms of the three different age groupings for every single species, treatment and sampling dates. Therefore, age groups were established by pooling the values of the different species, treatments and dates, and only the means per site are given (Fig. 5). The results showed that higher ^{13}C values were measured in the mature specimens than in the semi-mature ones at the Spanish site, whereas no significant differences were detected at the English site [Fig. 5(a) and (b)]. Earthworms in different age groups exhibited different N isotope ratios, with mature worms showing significantly higher ^{15}N values than immature ones at both sampling sites [Fig. 5(c) and (d)]. At the Spanish site mature worms were also significantly different from semi-mature ones.

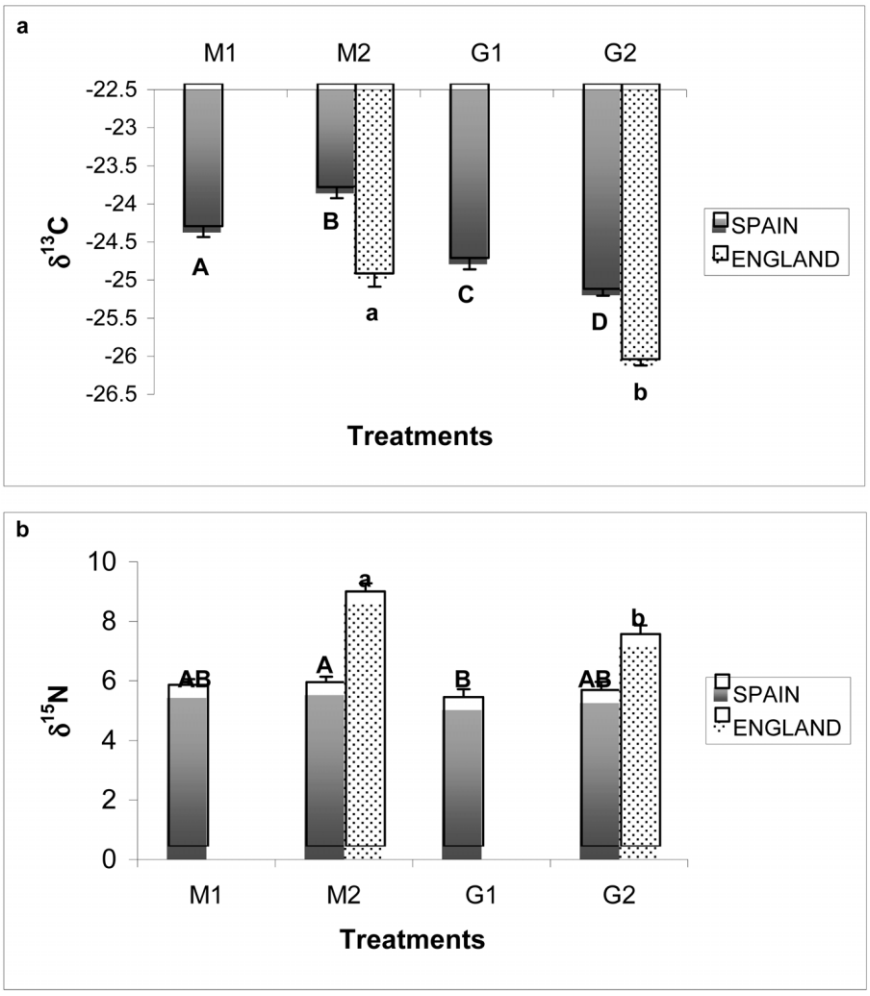

Fig. 2. $\delta^{13}C$ (a) and $\delta^{15}N$ (b) earthworm values from each treatment at both sites and results from one-way ANOVA with different letters indicating significant differences (capital letters showing significant differences between Spanish treatments and lower case letters showing significant differences between English ones). Abbreviations as in Fig. 1. Units are ‰.

4. Discussion

Intensification of agricultural practices is expected to occur as result of increasing demands for food supply and economic pressures (LUCC, 1999). This would lead to accelerated land-cover changes which in turn will greatly alter soil biodiversity and ecosystem function (GCTE, 1996).

Maize produces about 1.6 million tonnes of dry matter which is used as a supplement to livestock rations to help them to overcome the winter (Lister and Subak, 1999). It is an important crop which grows rapidly and yields more forage than ryegrass in warm conditions due to its highly effective photosynthesis mechanism. Its C_4 metabolism also leads to a difference in isotopic composition which allows rapid differentiation from C_3 plant tissues.

Stable isotope techniques have proved to be a powerful tool as tracer methodologies. They help to locate and quantify biological contribution to soil nutrient cycling and to identify metabolic fluxes between the different soil compartments (e.g. Boutton and Yamasaki, 1996). The combined use of C and N natural abundance stable isotope ratios has in some instances proved to be a more powerful tool than single isotope studies when studying feeding ecology (Spain and Le Feuvre, 1997; Neilson and Brown, 1999) or the competition between native and invasive exotic species (Hendrix et al., 1999a,b).

$\delta^{13}C$ values of organic matter reflect those of the dominant vegetation and they increase with soil depth as a result of isotopic fractionation during the decomposition processes (Balesdent et al., 1993; Boutton, 1996; Handley and Scrimgeour, 1997). Spanish soils were ^{13}C-enriched when compared to the English ones and this difference was also reflected in the earthworm tissues. This could be related to a more rapid turnover of the soil organic matter and a higher activity of the worm population in warmer climates. Earthworm activity depends on several factors, mainly climate, which directly affects their behaviour and life cycles, and indirectly influences their habitat and food supply (Curry, 1998).

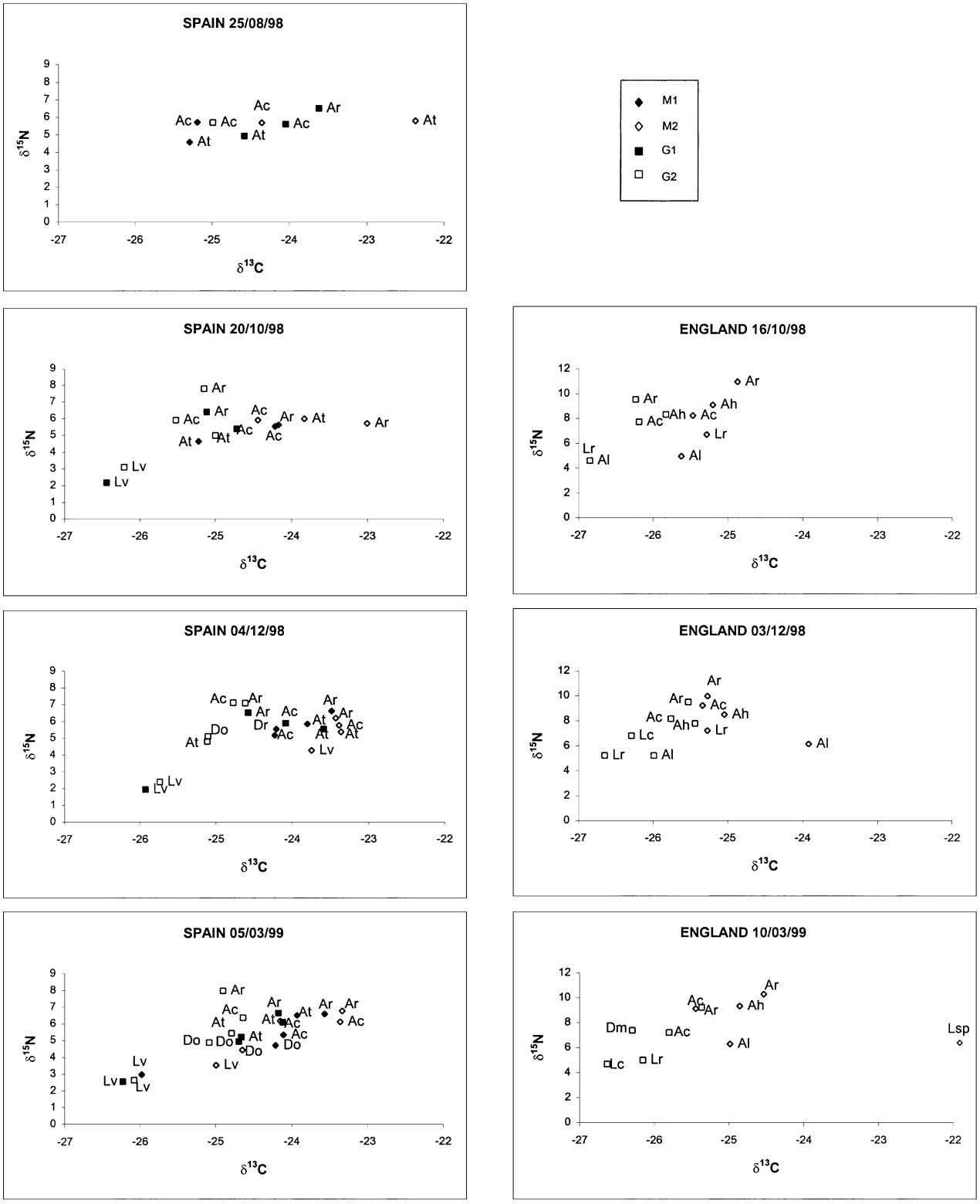

Fig. 3. $\delta^{13}C$ and $\delta^{15}N$ values for the earthworm species in the maize plots (M1 and M2) and in the grassland ones (G1 and G2) at the different dates at both sites. Treatment abbreviations as in Fig. 1. Species abbreviations: *Allolobophora caliginosa* (Ac), *A. rosea* (Ar), *A. trapezoides* (At), *A. chlorotica* (Ah), *A. longa* (Al), *L. rubellus* (Lr), *L. festivus* (Lv), *L. castaneus* (Lc) *Dendrobaena octaedra* (Do), *D. rubida* (Dr), *D. mammalis* (Dm). Units are ‰.

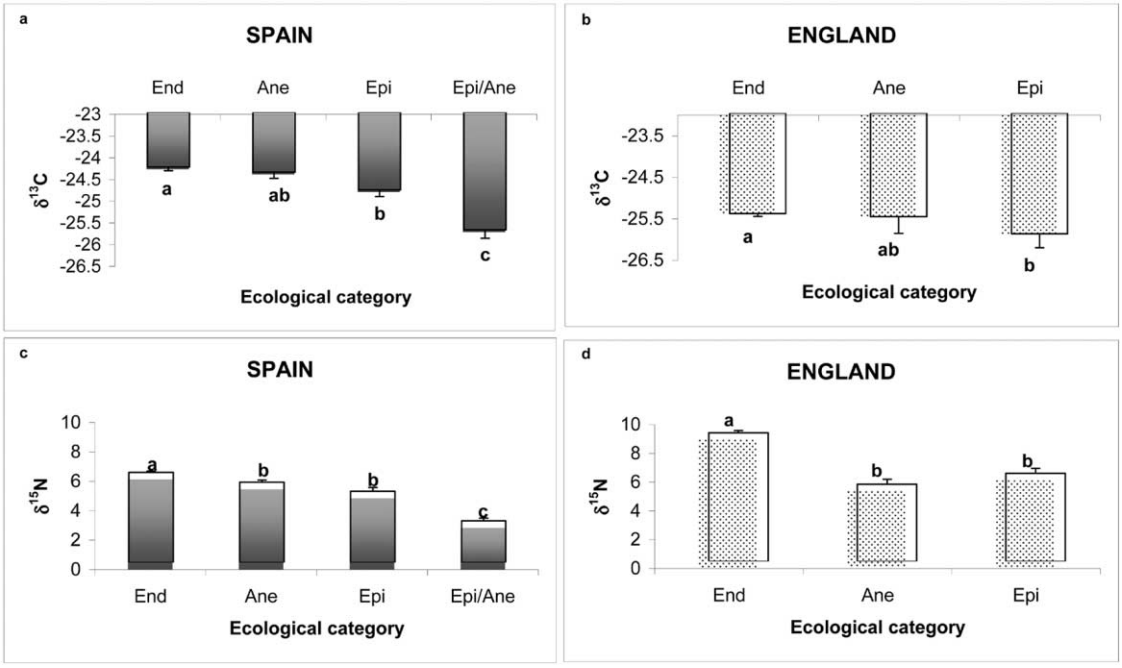

Fig. 4. $\delta^{13}C$ (a) and (b) and $\delta^{15}N$ (c) and (d) values of the different ecological categories of the earthworms at each site and results from one-way ANOVA with different letters indicating significant differences. Units are ‰.

At both sites $\delta^{13}C$ values became increased with time indicating that the worms were feeding on the maize, even after it had been harvested and after all the above-ground material had been removed from the field. The minor temporal isotope fluctuations in some species can be explained by more diversified feeding, probably due to different digestive capabilities (Lattaud et al., 1998), age (see below) or movements through the soil profile.

The enrichment in ^{15}N with soil depth, especially in the undisturbed grasslands, reflects both a more intense processing of the organic matter by microorganisms at deeper layers and a preferential loss of ^{14}N from the grassland system (Nadelhoffer and Fry, 1988; Balesdent et al., 1993). The higher ^{15}N values in the maize plots at both sites could be related to higher litter inputs of plant material, which are generally depleted in ^{15}N (relative to soil) in the soil under pasture.

The functional classification of earthworms reflects their burrowing abilities and life-history characteristics. Anecic

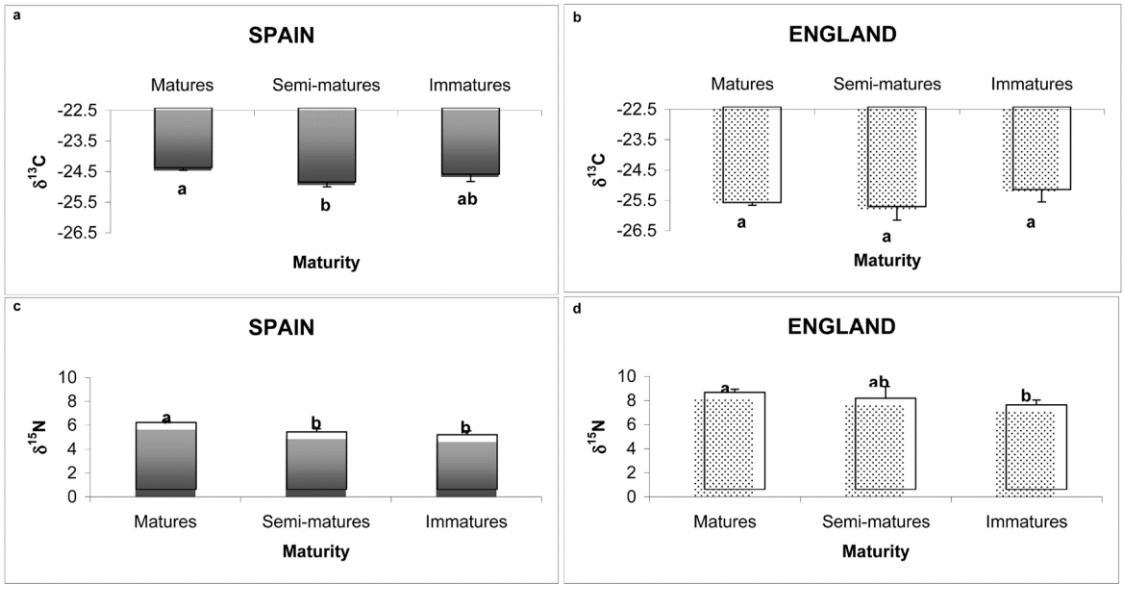

Fig. 5. $\delta^{13}C$ (a) and (b) and $\delta^{15}N$ (c) and (d) values of the different earthworm age groups at each site and results from one-way ANOVA with different letters indicating significant differences. Units are ‰.

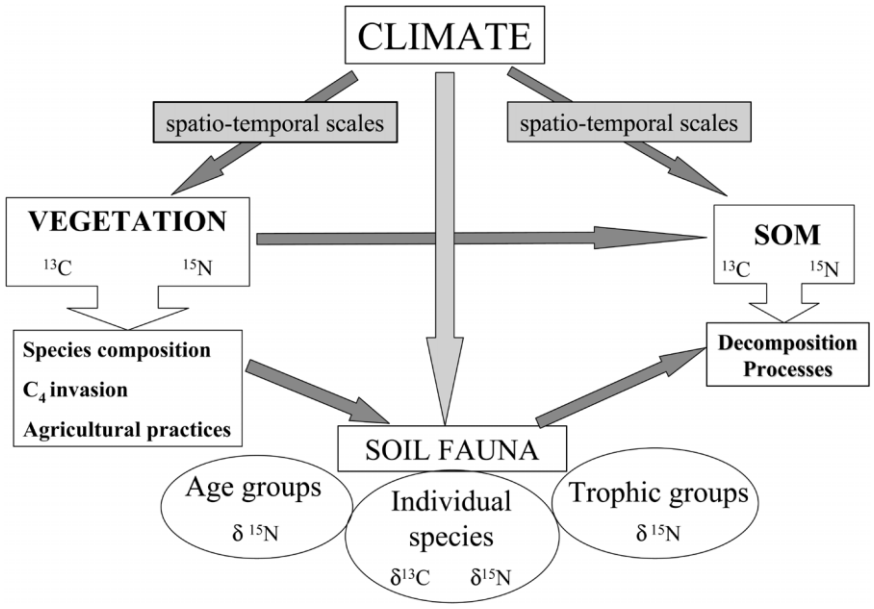

Fig. 6. Schematic diagram of the pathways of C and N isotopes in the soil system, showing the possibilities for tracing these transfers in each soil compartment.

and endogeic worms usually dominate the biomass in fertile temperate grasslands (Curry, 1994). They are less vulnerable to desiccation than the litter dwellers (epigeic worms) due to their ability to burrow to a greater depth and to aestivate. However, whereas endogeic worms are geophagous, anecic ones show a similar diet to the epigeic species, as it is reflected in their tissue $\delta^{15}N$ values. This has been used as an indicator of their trophic level (Schmidt et al., 1997; Hendrix et al., 1999a,b; Briones, et al. 1999a,b) and accordingly, in our work presented here, endogeic species showed the highest N isotope values. In contrast, no differences were detected between anecic and epigeic species as a result of selective feeding in deeper soil profiles by the former group.

Ontogenic changes can have an effect on $\delta^{15}N$ values (Ponsard and Averbuch, 1999). Schmidt et al. (1997) found significantly lower $\delta^{15}N$ values of juvenile *Aporrectodea longa* than those of the adults. Similarly, Hendrix et al. (1999b) found that adult *Pontoscolex corethrurus* showed a slight enrichment in ^{15}N relative to juveniles in the same population. In our study an increase in N isotope values was observed with increasing age which suggests that older animals feed on more microbially processed material, whereas young worms prefer fresh organic matter. This information emphasises the need for long-term studies and that life-cycles and juvenile emergence should be taken into account when performing isotopic analyses.

Seasonality strongly affects earthworm numbers. Reproduction peaks usually coincide with favourable moisture and temperature conditions in the soil which in temperate regions occur in spring and autumn. Neilson et al. (1998) observed seasonal changes in the δ values of earthworms which could be related to food source or animal behaviour changes. For example, they found minimum $\delta^{13}C$ and maximum $\delta^{15}N$ values in August when there was a deficit in soil moisture which could have affected earthworm activity. In our study sampling in the summer months was only carried out at the Spanish plots which at that time were very dry and the few specimens captured were aestivating suggesting that the population was inactive and therefore these results cannot be compared with previous work.

Climate not only has an important effect on soil faunal populations in terms of reproduction and activity, but also influences plant distribution which supplies fresh organic matter to soil biota. As human activities have been accelerating changes in land cover on a global scale (Turner et al., 1994; Houghton, 1994) it is expected that they will lead to changes in the composition of plant communities and to the invasion of exotic species. These changes in the vegetation cover will have an influence upon the amount, quality and distribution of soil organic matter, which will be reflected in the isotope composition. It is therefore necessary to improve our understanding of the effects of land use changes on soil ecosystems within spatial and temporal scales. A deeper knowledge of below-ground food webs is also essential to evaluate whole ecosystem responses to perturbation, and stable isotopes at natural levels can assist in examining the extent of human effects on ecosystem structure and function (Fig. 6).

Here we have exploited the existence of natural variations of carbon isotope values between maize (C_4) and grasslands (C_3) in order to be able to identify the preferential feeding material of the earthworms. Similarly, nitrogen isotope values have enabled us to determine trophic positions. We have also incorporated spatial (soil depth, different sites) and temporal scales (dynamic sampling) to the experimental set-up with the aim of gaining a deeper knowledge of isotopic fluctuations in the different soil compartments (Neilson et al., 1998; Briones et al., 1999a,b; Fogel and Tuross, 1999).

These kinds of isotopic approaches should be extended to

other soil organisms in a wide range of habitats, climates and land use practices to get a more complete picture of the role of soil biota in managed systems. This would result in important information about key organisms and key functions and would allow more sustainable soil and land management practices.

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