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Stable isotope techniques in studies of the ecological diversity and functions of earthworm communities in agricultural soils

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Abstract

Climate change is likely to alter agricultural landscapes leading to the conversion of large areas of grassland to arable land, the introduction of new crops and the intensification of production in currently cropped land. All of these developments will in turn affect the direction and magnitude of agricultural ecosystem responses to global change. The soil fauna in agricultural soils plays an important role in soil fertility and nutrient dynamics and earthworms in particular strongly influence soil properties and biogeochemical cycles. In order to develop more sustainable soil and land management practices, it is necessary to gain a better understanding of the biodiversity and functioning of soil animals and their response to land use changes.

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In this context, novel isotope techniques have proved to be powerful research tools for establishing food preferences and resource partitioning among soil invertebrates in general and earthworms in particular. In this paper, we review recent developments in the application of natural abundance carbon (C) and nitrogen (N) stable isotope ratio techniques in investigations of the ecological diversity and functions of earthworm communities in agricultural soils. The review includes a meta-analysis of published N isotope ratios in earthworms belonging to different ecological groups.

Introduction

Among global change drivers, land use is the main factor projected to impact on biodiversity by the year 2100 [1]. At the same time, agricultural practices have to increase productivity in order to feed a growing human population [2]. This will result in profound alterations of agricultural landscapes with the conversion of long-term grasslands to arable lands, the introduction of new crops and the intensification of production in currently cropped land. Climate change is expected to have a dramatic impact on arable land, with increasing temperatures and droughts having detrimental effects on crop yields. New crops better adapted to warmer conditions are likely to be introduced in temperate areas. For example maize cropping in the UK has increased due to an upward trend in air temperature since the mid 1960s, making it a good indicator of climate change [3].

The soil fauna in agricultural systems plays an important role in soil fertility and crop productivity. Earthworms (Annelida: Oligochaeta) in particular strongly influence soil properties and nutrient dynamics due to their feeding and burrowing activities and their interactions with other components of the soil system. However, compared to relatively undisturbed soils such as pastures, arable crops generally support smaller earthworm populations due to the mechanical impact associated with cultivation, the reduction of organic matter available as a food source, the loss of the insulating vegetation layer and the increase of predation by birds as the worms are brought to the surface. For example, estimated earthworm mortality rates due to direct injury caused by tillage range from 25% in various crops in Switzerland [4], 50% in potato crops in Australia [5], 60-70% in grass and lucerne leys in Sweden [6] to virtual elimination in intensively cultivated potato crops in Ireland [7].

In order to enhance the sustainability of agriculture, it is therefore necessary to develop management practices that ensure reduced disturbance and provision of sufficient food resources to support large and diverse earthworm communities in agricultural soils. Food supply appears to be a more important factor in earthworm population maintenance than the absence of disturbance and thus, populations might not increase if the food supply is restricted [8]. Furthermore, a better understanding is required of the feeding ecology of earthworms, the trophic relationships of the different species in communities and their likely response to land use changes.

Traditional feeding studies on soil animals rely on direct observation of the feeding activity, microscopic analyses of gut contents, palatability tests, estimates of ingestion and consumption rates, and measurements of the growth rates of animals feeding on different substrates. All of these methods are time consuming and have provided limited information on feeding strategies and assimilated dietary components. Recently, stable isotope techniques have been adopted by soil ecologists as a novel approach that is

independent of traditional methods. Natural abundance stable isotope techniques are based on the premise that the isotopic composition of animal tissues reflects that of the animal's diet. In research on soil animals, isotope ratios of carbon (expressed as δ^{13} C) and nitrogen (δ^{15} N) have been used most frequently while sulphur (δ^{34} S) is rarely used (e.g. [9]). This is in contrast with research on marine invertebrate ecology in which C and N isotope ratio measurements (e.g. [10,11,12,13]) have more often been combined very successfully with S measurements (e.g. [14,15,16]).

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Stable isotope techniques at natural abundance level are a powerful tool in soil animal ecology because they offer a means of studying undisturbed communities in different compartments of the soil system *in situ*. Most studies conducted to date have focused on the dietary preferences of selected groups of soil animals, including termites [17,18,19,20,21,22,23,24] and collembolans [25], but most research has concentrated on earthworms [26,27,28,29,30,31,32,33,34,35,36,37,38,39]. Furthermore, a number of investigations have focused on below-ground relationships among multiple soil animal groups [40,41,42] or in entire food webs specifically in forest soils [43,44]. This research on soil food web ecology has been reviewed recently by Scheu [45].

In this contribution we present an overview of recent advances in the development and application of natural abundance stable isotope ratio techniques in the investigation of the feeding ecology, trophic structure and role in elemental cycling of earthworm communities in agricultural soils. We first address some methodological aspects of stable isotope techniques which need to be considered in the experimental design, processing of samples for analyses and interpretation of results. As most attention in isotopic earthworm ecology studies has focused on feeding strategies and trophic structure, we then review the available literature on these topics. We present the results of a statistical meta-analysis of published N isotope ratios in earthworms belonging to different ecological groups and we discuss the suitability of this techniques with particular emphasis on the factors that can affect the isotopic composition of earthworms.

Methodological considerations

To interpret isotopic patterns in soil invertebrates successfully and reliably, we have to be aware that variations in natural abundance C and N isotope ratios in tissues of soil animals can have many causes. They can reflect assimilation of C and N from specific, isotopically different food sources which we can measure, but they can also be due to, for example, feeding on unknown food sources, physiological changes or biochemical body composition. Much more research is required to establish how factors like these affect the isotopic composition of soil invertebrates including earthworms and to validate experimentally the most important assumptions on which the interpretation of isotopic patterns are based.

One important factor is isotopic fractionation. This occurs between plants and the atmosphere, soil and plants, soil and biota and it is the result of preferential losses of one isotope over the other through assimilation reactions and metabolic processes [17,46,47,48]. In animals, fractionation leads to the observed discrepancy, or spacing, between the isotopic (in particular ¹⁵N) bulk value of the animal's body and its food. This effect and the associated step-wise enrichment in food chains have been documented and analysed extensively, most recently by McCutchan et al. [49], Olive et al. [50] and Vanderklift and Ponsard [51]. However, most of the available information

relates to higher vertebrate animals, while less is known about invertebrates and virtually nothing about soil invertebrates [45].

Several ontogenetic, physiological and biochemical factors can affect the isotopic composition of earthworms:

- (i) The absolute body size of an animal must be taken into account when interpreting its isotopic composition. In general, the isotopic composition of small animals is determined on whole animal bodies and gives an accurate estimate of the isotopic composition of their diet because tissue turnover is rapid [52]. In soils, this can be applied to the microfauna (nematodes, tardigrades, mites and collembolans) and mesofauna (enchytraeids). For macrofauna, including earthworms, it must be ensured that the subsamples which are analysed isotopically (typically 1 mg dry weight) are representative for the whole animal and the fact that tissues integrate dietary inputs over longer periods of time, because tissue turnover is slower [31], must be considered (see iii below). The contribution of the gut content, which can account for up to 30% of the animal dry weight in earthworms, must also be taken into account because the C and N contained in ingested, undigested gut content materials can adulterate the worms' bulk isotopic composition.
- (ii) Isotopic differences between different size or age classes of the same earthworm species have been interpreted as reflecting different diets [29,35]. However, it is not known whether metabolic processes can cause such isotopic differences between ontogenetic stages feeding on the same diet. For example, the ratio between respiration and reproduction can alter tissue δ^{13} C values in animals [53]. Large δ^{13} C differences between two discrete food sources have been exploited to demonstrate ontogenetic dietary shifts in a marine deposit-feeding polychaete [12].
- (iii) Another complicating factor is that seasonal changes in diet or in the diet's isotopic composition may occur. Seasonal variation in the isotopic composition of the diet can be considerable in plant-soil systems, making it necessary either to interpret an integrated average value cautiously or to analyse animal components as soon they are synthesised [52]. The isotopic signatures of earthworm tissues may integrate dietary signatures over shorter or longer periods of time depending on tissue turnover rates [49]. No data are available at present on the rates of turnover for different earthworm tissues. Depending on the development time of some earthworm species and the seasonality of their activity, whole-body isotopic signatures of adults could integrate the isotopic composition of their diet over several months.
- (iv) The lipid content of earthworms can affect C isotopic values because pyruvate deshydrogenase discrimination during lipid metabolism results in lipids being up to 10% lighter in δ^{13} C than other body tissues in animals [53,54,55,56]. An increase in lipid reserves has been observed in earthworms before entering diapause [57].
- (v) Body condition, nutritional stress or fasting have been shown to alter the isotopic composition of vertebrate animals [50,51,58], but the evidence of such effects in terrestrial invertebrates is incomplete [31,59,60].

Spatial variation in soil systems can also affect the isotope values in soil fauna and is mainly represented by soil depth, three-dimensional distribution of the rhizosphere,

and horizontal and vertical distribution of soil organisms. In undisturbed soils, C and N isotope values of bulk soil organic matter can increase with soil depth. Anecic earthworm species, which can burrow deep down the soil profile, are assumed to be feeding on surface litter [61], but feeding on deeper, isotopically enriched soil layers could also occur. Different size fractions of soil organic matter can also have different isotopic value compared to bulk soil or aggregates (e.g. [62,63,64,65,66,67,68]). Preferential feeding on certain soil organic matter size fractions by earthworms of different body sizes could therefore result in divergent isotopic tissue values. More generally, selective feeding by earthworms on certain particle sizes, biochemical fractions, casts or microorganisms can result in different isotope values, which, in the absence of additional information on likely feeding habits, can make interpretations very difficult. Since food sources are usually measured as 'bulk' samples (all carbon and nitrogen-containing molecules are averaged), selective assimilation of certain dietary components can result in unexpected diet–tissue spacings [51].

To clarify changes in specific diets and soil organic pools in spite of inherent variability and fractionation effects described before, it is necessary to establish whether the isotopic signatures of food source are sufficiently different. The power of isotopic techniques is increased when carbon and nitrogen isotope measurements are combined in soil ecological studies when diets have overlapping isotopic ranges in one element [28,29,30,32,33,34,35,36,37,38,39,69].

Another important methodological decision is whether to perform the isotope analyses on pooled samples of many individuals or on separate individuals. Since earthworms are ecologically diverse, pooling of species is in our opinion not advisable. Isotope ratios of pooled animals of the same species give sufficient information to detect differences between isotopically clearly distinct treatments and can be a suitable method when analysing animals cultured in the lab on a standard diet. However, they have the disadvantage of obscuring the underlying individual variability, even if several "replicates" are analysed. Most information can be gained from individual samples when one individual of a single species is treated as a true replicate. Furthermore, different fractions of the same specimen can be analysed, for instance whole body and mucus. As C and N in mucus are replaced more rapidly than that of other body tissues, separate tissue and mucus analyses may provide short-term and long-term information on dietary inputs [29,31,70].

Lancaster and Waldron [71] have concluded from a detailed methodological study on freshwater macro-invertebrates that the optimum number of replicate samples depends on the hypotheses of interest and that pre-experimental estimates of withinpopulation variation is required to optimise sampling programmes in terms of sampling effort, analytical cost and statistical power. These authors recommend to decrease the number of species or groups under investigation rather than compromise the statistically validity of the results. Since earthworm communities in agricultural soils can comprise 10 and more species (e.g. [8,72]), pre-experimental estimates of variability and careful planning are required to achieve the objectives of the study. A high number of samples to be analysed will result from multiplying number of species (or ecological groups) by number of replicates for each species, which could be increased further by taking replicate samples of different age groups.

Tracing dietary changes

Since earthworms are detritivores, their gut contains a mixture of different mineral and organic materials, plant residues and microorganisms. It is therefore very difficult to identify the main components of their diet, especially the components that are digested and assimilated. One novel approach for the identification of food sources assimilated by earthworms exploits natural differences in stable isotope ratios between food sources. One of the most useful isotopic source difference at natural abundance level that has been exploited successfully in terrestrial ecology and soil science is that between plants using the C₃ and C₄ photosynthetic pathways. The existing differences in C isotopic composition between plants and atmosphere which are a function of enzyme discrimination, diffusion and dissolution processes and metabolic reactions, result in a distinctive isotopic composition of the two plant groups with C₃ plants ranging from –20 to $-35\% \delta^{13}$ C and C₄ plants ranging from –9 to $-14\% \delta^{13}$ C [73,74].

Agricultural systems containing C_3 and C_4 plants can be used to investigate the effect of earthworms in reducing the great volume of crop residues which are left on the land after harvesting. For example, it is now a common agricultural practice in Australia to retain harvest residues from sugarcane plantations (C_4) as surface mulch or as a green manure [75]. By analysing $\delta^{13}C$ and $\delta^{15}N$ values of sugar cane tissues, harvest residues, soil and casts and body tissues of the earthworm *Pontoscolex corethrurus*, Spain and Le Feuvre [28] concluded that this earthworm species feeds mainly on organic matter derived from the roots and from the decomposing harvest residues. This finding agrees with the widely accepted view that many earthworm species benefit from an ample supply of new organic matter.

A similar approach was pursued in tropical soils where crops are dominated by Gramineae (mainly C_4 plants) and where geophagous earthworms including *Millsonia* anomala play an important role in C turnover [76]. By feeding earthworms collected from C_4 -dominated soils with C_3 organic matter, which provided a natural isotopic C label which could be traced, Martin et al. [26] showed that *M. anomala* is able to assimilate C from fresh plant debris, coarse organic matter (>50 µm) as well as some fine soil fractions.

Natural C isotope ratios can also be used to detect changes in the relative dominance of C_3/C_4 plant species and how habitat changes impact on the feeding behaviour of earthworm communities. For example, in the Ivory Coast the protection of savannas (C_4 grasses) from fire and the rapid re-colonisation by trees (C_3) resulted in a natural C isotope labelling of soil organic matter [62]. After 19 years of this vegetation shift, the isotopic composition of the earthworm populations showed that nearly 100% of the soil C assimilated by earthworms was derived from C_3 plants [27].

Other studies have been conducted on the effects on earthworm populations of crop conversions in temperate regions from long term grassland (C_3) to continuous maize (C_4). By measuring the distribution of C_3 -C and C_4 -C in the earthworm tissues in French maize fields, Martin et al. [27] concluded that irrespectively of their ecological grouping, all earthworm species examined mainly fed on recent soil organic matter pools that are more easily decomposable. Similar studies in Spain and England [33,34] showed that even after the maize had been harvested and removed from the field, earthworms still fed on maize residues (probably decomposing roots), but there was a large contribution of the older native C_3 material predating the conversion to maize. However when temporal

scales where included in the study, by simulating crop rotations, earthworms exhibited selective assimilation of fresh C_4 -C from residue over older native C_3 [35].

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When the vegetation systems under investigation do not have distinct signatures in their carbon sources, N isotope values can sometimes be used to determine N resource partitioning among earthworm species. This was particularly useful in subtropical savannas where earthworms show a generalist feeding strategy although they tend to feed preferentially on the most abundant carbon input [37]. Variations in the δ^{15} N values could therefore allow a better discrimination of food sources, including potential competition, as a result of exotic species invasion. For instance, Hendrix et al. [38] hypothesized that overlapping N isotope ratios could reflect interspecific competition, whereas distinct N enrichment in the earthworm tissues indicates different food sources and absence of competitive interactions between native and exotic species in Puerto Rican forest and pasture systems.

Consistent differences in N isotope ratios between atmospheric N_2 and plantavailable soil N can also sometimes be used to estimate the relative contribution of atmospheric N_2 to N_2 -fixing plants [77] and to identify N sources assimilated by soil animals. Temperate bicropping systems, in which legumes (with N_2 -fixing rhizobial symbionts) are cultivated in mixture with cereals (without the symbiotic association), contain large earthworm communities as a result of continuous supply of high quantity and quality food throughout the year [72]. By comparing natural N isotope signatures in earthworms from conventional wheat monocrops with those from wheat-clover bicrops, Schmidt et al. [30] showed that the seven species analysed derived some of their N from clover N in the bicropping system.

Schmidt and Ostle [78] exploited the fact that animal manures can be naturally enriched in ¹⁵N to identify N sources in the diet of earthworms and concluded from a field experiment in England that the two earthworm species studied assimilate N derived from slurry. This suggests that δ^{15} N values can potentially be employed to trace anthropogenic inputs (e.g. organic fertilisers, sewage) through soil food webs, as they have been used successfully to track such inputs in freshwater and marine biota (e.g. [11,15].

In conclusion, these studies suggest that most of the earthworm C and N nutrition is derived from recent residues and organic matter inputs, and that natural abundance isotope techniques are useful in investigating the change in feeding behaviour of earthworm populations under different cropping systems. However, variations in the δ^{13} C and δ^{15} N spacing between earthworms and their food sources have been observed across different habitats and sampling periods and in response to physiological and biological changes which can obfuscate simple interpretations. As stated earlier, several factors can affect isotope fractionation and therefore more research is needed for a full interpretation of isotopic measurements.

Investigating feeding guilds

Ecological groups of earthworms comprise different species which obtain their nutrition from similar sources. Species belonging to the same group are assumed to be functionally equivalent and to play a similar role in the organic matter turnover. Although several ecological classifications have been proposed [79,80,81,82], the most widely accepted one is that by Bouché [61] which distinguishes three main groups differing, among other characteristics, in their feeding behaviour: (i) *epigeic*: heavily

pigmented worms which live in and feed on fresh organic matter in soil surface layers; (ii) *anecic*: partly pigmented worms which construct vertical burrows and feed on decomposing litter at the soil surface, and (iii) *endogeic*: barely pigmented worms feeding on more decomposed soil organic matter fractions.

Residual and decomposed plant material and hence soil organic matter in agricultural systems are often isotopically enriched compared to the original plant material from which they are derived because the decomposition and microbial processing of plant litter involves several processes that can be associated with isotopic fractionation (e.g. [83,84]). Depending on the food sources earthworms use, these isotopic source differences between fresh and microbially transformed plant materials may translate into isotopic differences in earthworm tissues. Schmidt et al. [30] recorded, for the first time, that tissue δ^{15} N values of earthworms reflect ecological groupings; δ^{15} N values were highest in endogeic species followed by anecics and epigeics. Several workers have subsequently tried to relate the N isotopic composition of different earthworm species to feeding habits, generally finding similar results at different sites [35,36,37,38,42,44]. However, Neilson et al. [32] argued that this functional classification is the result of habitat diversity and not related to different feeding activities.

In order to establish weighted comparisons between the different N isotope values recorded for different earthworm species at different sites and geographic locations, we have used meta-analysis [85] to synthesize available data. In this formal statistical approach individual reports are collated and compared statistically in order to describe generalizable patterns from the findings in the selected set of studies [86]. Our dataset comprises available information on N isotope ratio measurements in earthworms collected from agricultural soils (Table 1). Complementary information was also compiled from the publications regarding the site location (longitude and latitude), number of soil samples collected, crop, earthworm species under study and their ecological grouping. Only data based on replicated, species-specific earthworm collections and analyses (i.e. isotopic analyses being performed on individual species with a minimum number of two independent replicates) were included. Because this statistical tool can only incorporate results reported with an associated error term and sample sizes, a few valuable studies could not be included, but still a reasonable set of data was available for meta-analysis (Table 1). Data originated from experiments in Australia, Germany, Ireland, Spain and Great Britain. All earthworms included in the dataset apart from Pontoscolex corethrurus (Glossoscolecidae) belong to the family Lumbricidae. Since taxonomic debate on the Allolobophora/Aporrectodea and Satchellius/Dendrobaena genera is ongoing, species names are listed and cited here as they were published in the original papers.

We conducted a meta-analysis to obtain weighted comparisons of N isotope values for different earthworm species when they are grouped according to trophic guilds. Results are reported graphically showing the 'effect size' value together with the confidence intervals (Fig. 1). Arithmetic means are used as the effect size statistic in order to give a quantitative statistical information from each research finding [86].

The meta-analysis reveals that N isotope ratios of endogeic worms are clearly distinct from those of the other ecological groups, showing a significantly higher enrichment in the endogeic, soil-feeding group than in guilds that feed mainly on plant litter. In addition, the

 Table 1. References used for the meta-analysis of N isotope ratios in earthworms belonging to different ecological groups.

| | | | | ~ | Ecological | a . |
|-------------------------------------------------|----------------------|------------------------------------------------|------------------------------------------------|-------------------------------------------------------------------------------------------------|-----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Author | Site | Latitude | Longitude | Сгор | grouping | Species |
| Spain and Le Feuvre 1997 Schmidt et al. 1997 | Australia Ireland | 17 ⁰ 30' S 53 ⁰ 18' N | 146 ⁰ 04' E 6 ⁰ 33' W | Sugarcane Wheat | END END | Pontoscolex corethrurus Aporrectodea caliginosa Allalabanhara chlaratica |
| | | | | Wheat-clover | ANE EPIANE END END ANE END EPIANE | Alloboophin chaotal Aporrectodea longa Lumbricus spp. Aporrectodea caliginosa Alloboophora chlorotica Allobophora longa Aporrectodea rosea Lumbricus spp. |
| Schmidt and Ostle 1999 | United Kingdom | 50 ⁰ 48' N | 4 ⁰ 38' W | Wheat-clover | END ANE | Aporrectodea caliginosa Aporrectodea longa |
| Schmidt 1999 | Ireland | 52 ⁰ 84' N | 6 ⁰ 92' W | White clover | ANE | Aporrectodea longa |
| Neilson et al. 2000 | United Kingdom | 56 ⁰ 29' N | 3 ⁰ 04' W | Wheat | END END EPI EPI ANE | Aporrectodea caliginosa Allolobophora chlorotica Lumbricus castaneus Lumbricus rubellus Lumbricus terrestris |
| | | 56 ⁰ 18' N | 3 ⁰ 39' W | Grasses | END END END ANE | Allolobophora chlorotica Aporrectodea rosea Octolasion cyaneum Lumbricus terrestris |
| Briones et al. 2001 | Spain | 42 ⁰ 53' N | 8 ⁰ 27' W | Maize | END END ANE EPI EPIANE | Allolobophora caliginosa Allolobophora rosea Allolobophora trapezoides Dendrobaena octaedra Lumbricus festivus |
| | United Kingdom | 50 ⁰ 48' N | 4 ⁰ 38' W | Maize | END END END ANE EPI | Allolobophora caliginosa Allolobophora chlorotica Allolobophora rosea Allolobophora longa Lumbricus rubellus |
| Briones and Bol in press | United Kingdom | 50 ⁰ 48' N | 4 ⁰ 38' W | Bi-crop old | END END ANE | Allolobophora caliginosa Allolobophora chlorotica Allolobophora longa |
| | | | | Bi-crop new | ANE END END ANE EPI EPI ANE | Lambricus terrestris Allolobophora caliginosa Allolobophora chlorotica Allolobophora longa Lumbricus castaneus Lumbricus terrestris |
| | | | | Clover | END END END ANE | Allolobophora caliginosa Allolobophora chlorotica Allolobophora rosea Allolobophora longa Lumhicus tarrastris |
| | | | | Maize | END END END ANE | Allolobophora caliginosa Allolobophora chlorotica Allolobophora tosea Allolobophora longa Lumbicus torrestris |
| Schmidt et al. unpublish | Ireland | 53 ⁰ 18' N | 6 ⁰ 33' W | Barley | END END END ANE EPIANE END EPI | Lambrius Peresiris Aporectodea caliginosa Allolobophora chlorotica Allolobophora tonga Lumbricus spp. Murchieona minuscula Satchellius mammalis |
| Albers 2002 | Germany | 51 ⁰ 29' N | 9 ⁰ 56' W | Winter rye Winter rape Maize Winter rye Maize Winter rye Winter rape Maize | END END END END ANE ANE ANE | Aporrectodea caliginosa Aporrectodea caliginosa Aporrectodea caliginosa Aporrectodea rosea Aporrectodea rosea Lumbricus terrestris Lumbricus terrestris Lumbricus terrestris |

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Figure 1. Results of the meta-analysis showing the Grand mean effect size (closed circle) and the mean effect sizes for earthworm ecological groups (E_+ , open circle) together with 95% confidence intervals.

analysis detected significant differences between epigeic, epianecic and anecic worms with the latter having the lowest N isotope values.

In order to test whether ontogenic changes have an effect on N isotope ratios, the same analysis was repeated with those studies only in which information on the maturity of each species was given. Consequently six studies (2, 4, 6, 7, 8 and 9 from Table 1) were included in the analysis and the results for mature and immature worms are shown in Fig. 2.

Again, endogeic species had the highest ¹⁵N enrichment in both mature and immature worms. However, in the case of mature worms, significantly lower values were detected in anecic worms when compared to epigeic ones (Fig. 2a). No significant differences from the mean were observed for epianecic immature worms which also showed an N isotope ratio similar to that of anecic species (Fig. 2b). Careful interpretation of these results is required as the epianecic category as used here includes immature individuals of the genus *Lumbricus* which, as far as adults are concerned, comprises both epigeic and anecic species. Furthermore, only those studies performed in agricultural soils were included in this analysis and therefore further studies need to be incorporated for a full interpretation of food partitioning among earthworm ecological groups. However, the weighted comparisons presented here strongly suggest that earthworms have selective feeding behaviours in agricultural systems and therefore, this could enable them to avoid competition for the same food resources.

Since soil organic matter (SOM) is distributed in an extremely heterogeneous environment which offers a great variety of food sources, different earthworm species process organic matter in different ways leading to the well established ecological classification [61]. However, isotope tracer work has shown that endogeic and epigeic species can obtain their carbon nutrition from the same litter source [87]. This could be related to the fact that species within the same ecological group can assimilate different





Figure 2. Results of the meta-analysis showing the Grand mean effect size (closed circle) and the mean effect sizes for earthworm ecological groups (E_{+} , open circle) together with 95% confidence intervals for (a) mature and (b) immature worms.

sources under different environmental conditions [26]. This could explain observed changes in natural abundance C and N isotopic composition of the different trophic compartments with seasons [40]. In contrast, our results from meta-analysis are in agreement with the established functional classification, at least for mature worms. We therefore conclude that the N isotopic composition of earthworm tissues can provide a reliable estimate of feeding strategies.

In conclusion, natural abundance stable isotope techniques are one of the most promising tools for determining how earthworm populations and other soil invertebrates respond to land use changes at both temporal and spatial scales. They can supply novel, independent information about key organisms and key functions in a wide range of

habitats and climates. To gain a better understanding of nutrient cycling processes in agricultural systems, this techniques should be applied to other soil animals, other components of the soil system and entire below-ground food webs, as has been attempted twice in forest systems [43,44]. Only one such soil food web study has been conducted in an agroecosystem [41] form which it was concluded that generalist predators are potential biocontrol agents due to the strong link between detrital and grazing foodwebs. Future research on the interactions between the different food web components, including earthworms, will assist us in the development of more sustainable soil and land management practices and will help us to understand the interrelationships between global change, soil biodiversity and nutrient cycling [88]. Stable isotope techniques can potentially also be used to evaluate the response of agricultural ecosystems to perturbation and to provide an estimate of human effects on ecosystem structure and function.

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