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Short communication

Earthworm ecological groupings based on ^{14}C analysis

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Abstract

We report the first use of ^{14}C isotope analysis to investigate the ecological grouping of earthworms. Mature endogeic (*Allolobophora caliginosa*), mature epigeic (*Lumbricus rubellus*), and semimature anecic worms (*A. longa*) were collected in September 2002 from a woodland site at Lancaster, UK. Because anecic worms are known to have a variable feeding behaviour and can show dietary changes during ontogeny, additional immature and mature specimens of *A. longa* were also collected from the same site in January 2004. Epigeic earthworms showed the lowest radiocarbon concentration (0–3-years old), implying that they assimilated more recently fixed carbon than the anecic or endogeic earthworms. The age of carbon assimilated in mature anecic species (5–7-years old) was closer to that of endogeic species (5–8-years old) than to epigeics, suggesting that a greater proportion of older, more mineralised organic matter may form part of the diet of the anecic earthworms than previously thought. These results suggest that ^{14}C approaches are useful in the study of the feeding behaviour of detritivorous animals by providing in situ information on the age of the carbon assimilated by the worms. This can then be related to their role in ecosystem functioning, particularly in carbon cycling.

Keywords: Earthworms; ^{14}C-bomb; Ecological categories; Carbon

Several schemes have been proposed to classify earthworm species into ecological categories, mainly based on the burrowing activities and feeding behaviour as well as morphological and physiological characteristics of earthworms. The most widely used classification has been proposed by Bouché (1971, 1972, 1977), who recognised three ecological groups, ‘epigeics’, ‘endogeics’ and ‘anecics’, among European lumbricids. Epigeic worms are surface active, pigmented non-burrowing worms with relatively high reproductive rates which consume decaying plant residues on the soil surface; anecic worms build vertical burrows in the soil which descend into the mineral horizons but they feed at the surface usually at night; endogeic worms inhabit the organo-mineral and deep horizons, constructing branching sub- and horizontal burrows and they feed on more humified organic matter.

These major ecological groups could well represent functional adaptations to the soil environment that allow different species of earthworms to coexist by exploiting different food sources and habitat space (Edwards and Bohlen, 1996). Other authors question this interpretation, arguing that earthworm ecological groupings are indicative of habitat diversity and not related to different feeding activities (Neilson et al., 2000).

Traditional techniques used to determine earthworm food sources (direct observation of feeding activity, microbiological analysis of gut contents, palatability tests, recording ingestion rates and measurements of the growth rates of the worms growing on different substrates) are time-consuming. More recently, stable isotope techniques have been used to determine food sources. Isotope techniques have the advantage of providing an accurate estimate of the δ^{13}C value of the animal’s diet because of the short time period for assimilation of dietary carbon (DeNiro and Epstein, 1978). There is a direct relationship between N isotope ratios and the ecological groups of earthworms; Schmidt et al. (1997) observed δ^{15}N values in the order epigeic < anecic < endogeic, results confirmed by later work (reviewed by Briones and Schmidt, 2004) which showed

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that endogeics had higher $\delta^{15}N$ values than epigeics, suggesting that they fed on more mineralised material. In contrast, anecic worms showed more isotopic variation as a result of their vertical movements through the soil profile but were often close to the epigeics as a result of their putative feeding on plant litter.

Although stable isotopes have proven to be powerful tools in detecting trophic shifts, problems arise in relation to fractionation effects due to selective incorporation of the heavier isotope through the food chain (e.g. McCutchan et al., 2003). This has led to the observed discrepancy between the isotopic value of the animal's body and its food. In the case of earthworms, several factors can affect this preferential loss of one isotope over the other. They include body size (DeNiro and Epstein, 1978), body condition as nutritional stress or fasting (e.g. Vanderklift and Ponsard, 2003), lipid content of the body (e.g. DeNiro and Epstein, 1977), ontogeny changes (Schmidt, 1999; Briones et al., 2001), vertical distribution (Briones et al., 2001) and seasonal changes in diet or in the diet's isotopic composition (DeNiro and Epstein, 1978; see also review by Briones and Schmidt, 2004). These aspects introduce uncertainty when interpreting the results in terms of earthworms' classification.

In contrast, radiocarbon approaches provide in situ information on the age of the carbon assimilated by the worms (Briones and Ineson, 2002), and have the advantage that no disturbance of the habitat is required and the potential dietary materials do not have to include changes in $\delta^{13}C$ values between C_3 and C_4 plant carbon sources (Tayasu et al., 2002). ^{14}C dating has proven to be a successful tool in tracing carbon intake in vertebrates (e.g. Beavan and Sparks, 1998; Beavan-Athfield and Sparks, 2001a,b), termites (Tayasu et al., 2002) and enchytraeids (Briones and Ineson, 2002). In view of this, in this study we used radiocarbon analysis to investigate the ecological grouping of earthworms for the first time. The results will enable us to refine earthworm ecological classifications and better understand the role that earthworms play in the cycling of soil carbon.

The earthworms were collected from a woodland site at Lancaster University campus (54°1′N, 2°47′W). Sampling at the site revealed that the earthworm community was dominated by endogeic species although anecic worms accounted for most of the biomass. Specimens of endogeic (*Allolobophora caliginosa* (Savigny)), epigeic (*Lumbricus rubellus* Hoffmeister), and anecic (*A. longa* Ude) species were collected by hand-sorting in September 2002. Three mature specimens of the former two species and three semimature ones of the anecic worm were individually prepared for ^{14}C analyses (Briones and Ineson, 2002). Because anecic worms tend to have a more variable feeding behaviour than other species, and in particular, *A. longa* can show dietary changes during ontogeny (Schmidt et al., 1997), immature and mature specimens of *A. longa* were also collected from the same site in January 2004. Samples of living vegetation at the study site were also prepared for ^{14}C isotope analysis (Harkness and Wilson, 1972).

No pre-treatment of the samples was required and their preparation prior to isotopic analysis was carried out at the NERC Radiocarbon Laboratory (NERC RCL, East Kilbride, UK). Worm samples were analysed for ^{14}C concentration by accelerator mass spectrometry (AMS) analysis (Xu et al., 2004) and, vegetation samples by conventional radiometric analysis (Harkness and Wilson, 1972). For AMS, the total carbon in a known weight of the raw samples was recovered as CO_2 by combustion with CuO in a sealed quartz tube (in a muffle furnace over 12 h, with temperature peak of 900 °C). The gas was recovered and purified by selective cryogenic trapping (Boutton et al., 1983) and then converted to an iron/graphite mixture by Fe/Zn reduction (Slota et al., 1987) followed by preparation as accelerator targets for ^{14}C measurement at the Scottish Universities Environmental Research Centre's 5 MV AMS (SUERC AMS, East Kilbride, UK; for sample reference, see Publication codes in Table 1). Samples for radiometric ^{14}C analysis were converted to benzene and measured on a liquid scintillation counter at the NERC Radiocarbon Laboratory (NERC RCL, East Kilbride, UK).

Radiocarbon concentrations were expressed as % modern after normalisation of the measured ^{14}C enrichment of each sample relative to $\delta^{13}C_{PDB} = -25‰$ (Stuiver and Polach, 1977; Donahue et al., 1990). The calendar age of the earthworm carbon since fixation was determined by comparing the worm ^{14}C contents to an annual record of recent atmospheric ^{14}C concentration (Schauinsland site, Levin and Kromer, 2004). However, it was found that the ^{14}C content of the vegetation samples was slightly higher (by 1.74% modern) than the atmospheric ^{14}C value for the sampling year provided by Levin and Kromer. Since the vegetation samples we analysed were grown in the sampling year, the results suggest that there may be a small local or regional difference in atmospheric ^{14}C concentrations between our site and the continental European site used by Levin and Kromer (47°55′N, 7°55′E). This difference could be due to different levels of fossil fuel-derived CO_2 in the atmosphere, since Levin and Kromer consider that the atmospheric ^{14}C signal at their Schauinsland site is depleted by fossil CO_2 pollution. However, being located near the western coast of the UK, the Lancaster site is less likely to be influenced by pollution and should therefore have a $^{14}CO_2$ levels closer to the marine background (e.g. as at Mace Head, west coast of Ireland, 53°19′N, 9°53′W; Levin and Kromer, 2004) which would explain the slightly higher ^{14}C concentrations in the vegetation samples. For this reason and based on the ^{14}C results of the vegetation samples, we added 1.74% modern to the Levin and Kromer values to produce an atmospheric ^{14}C record for the Lancaster site.

Fig. 1 shows the variation in ^{14}C content of the earthworm species and Table 1 provides the ages of the samples following calibration with the atmospheric ^{14}C

Table 1
Publication codes, ^{14}C enrichment values (±SD), calibrated ages and collection date for each of the earthworm species

Publication code SUERC-	Species	^{14}C enrichment (%mod ± 1σ)	Calibrated age (y)	Collection date (y)
556	*L. rubellus* (M)	109.37 ± 0.93	2002	2002
557	*L. rubellus* (M)	110.67 ± 0.71	2001	2002
2566	*L. rubellus* (M)	111.39 ± 0.39	1999	2002
1553	*A. caliginosa* (M)	112.33 ± 0.39	1997	2002
562	*A. caliginosa* (M)	113.93 ± 0.88	1994	2002
2567	*A. caliginosa* (M)	113.05 ± 0.33	1995	2002
558	*A. longa* (SM)	113.96 ± 0.56	1994	2002
559	*A. longa* (SM)	111.85 ± 0.68	1998	2002
2568	*A. longa* (SM)	113.56 ± 0.52	1995	2002
2569	*A. longa* (M)	111.43 ± 0.39	1999	2004
2570	*A. longa* (M)	112.26 ± 0.40	1997	2004
2572	*A. longa* (M)	111.81 ± 0.39	1998	2004
2573	*A. longa* (I)	111.13 ± 0.32	2000	2004
2576	*A. longa* (I)	111.01 ± 0.36	2000	2004
2577	*A. longa* (I)	111.84 ± 0.35	1998	2004

Abbreviations: M, mature, SM, semi-mature, I, immature.

record. The results for *L. rubellus* indicate that this species assimilates more recently fixed carbon (0–3-years old) than the anecic or endogeic earthworms. ^{14}C results also showed that the C assimilation of the anecic earthworm species (5–7-years for mature worms) is similar to that of the endogeic worms (5–8-years old).

^{14}C assimilation in *A. longa* of different ages showed some slight differences, with semimature worms assimilating the oldest carbon substrates (4–8-years old) compared to the juvenile worms (4–6-years old). This suggests that there may be dietary changes with ontogeny although the differences were not significant (Fig. 1).

From the above, it seems that a functional classification applies to earthworms and that different species feed on C of different age, with epigeic earthworms assimilating more recently fixed carbon than the anecic or endogeic earthworms. This confirms previous findings (Bouché, 1972) which have suggested that epigeic worms consume relatively fresh decaying plant residues (they inhabit the surface organic layers of soil). The results for the endogeic species are also in agreement with existing classifications (Bouché, 1972), showing that these earthworms assimilate older carbon sources (on average c. 7 years relative to fixation from the atmosphere) compared to the epigeic worms.

Furthermore, the ^{14}C results suggest that the carbon assimilated in anecic species is of more similar age to that of endogeic species than to epigeics. This is surprising since anecic worms are known to feed on surface litter (Bouché, 1972), and therefore it was anticipated that they would preferentially assimilate more recently fixed C, similar to epigeic species. However, these preliminary ^{14}C results suggest that a greater proportion of older organic matter may form part of the diet of the anecic earthworms than previously thought. One possible explanation is that these worms have a more variable feeding behaviour compared to other species (Schmidt et al., 1997). It is known that

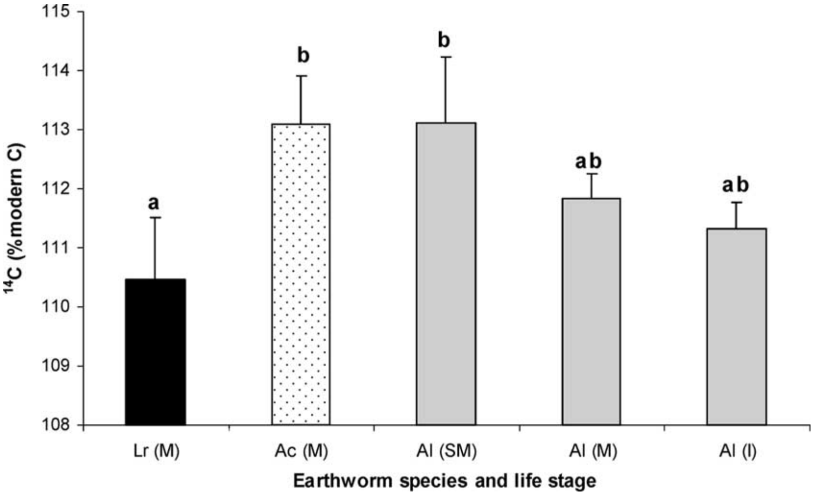

Fig. 1. ^{14}C enrichment in three earthworm species: *Lumbricus rubellus* (mature worms) = Lr (M), *Allolobophora caliginosa* (mature worms) = Ac (M), *A. longa* (semi-mature worms) = Al (SM), *A. longa* (mature worms) = Al (M), *A. longa* (immature worms) = Al (I). Mean values with SD and results from one-way ANOVA with different letters indicating significant differences (Tukey grouping, $P < 0.05$).

A. longa ingests substantial amounts of soil organic matter as a result of burrowing activities, i.e. it is an intermediate soil-litter feeder (Satchell, 1980).

Another explanation for the observed results is related to dietary changes occurring during ontogeny (Schmidt et al., 1997). Although juvenile worms are considered to inhabit surface layers of soil, adults of *A. longa* have been found as deep as 50 cm in the soil (Gerard, 1967); whether they could obtain their carbon sources from these deeper layers is uncertain. In relation to this, previous research using stable isotopes has shown that growing animals and adults can have different diets, affecting the N isotope composition of the animal tissues (Hendrix et al., 1999; Schmidt, 1999; Briones et al., 2001; Briones and Bol, 2003; Briones and Schmidt, 2004). However, in our study no significant differences were detected in the ^{14}C enrichment between juvenile worms and adults of *A. longa*.

A third explanation is that climatic factors might cause species within the same ecological group to assimilate C from different sources under different environmental conditions (Martin et al., 1992). This could explain observed changes in natural abundance C and N isotopic composition of the different trophic compartments with season (Neilson et al., 1998). For example, our semi-mature specimens of *A. longa* were collected during the first sampling in September 2002, a dry month with a total rainfall of 50.6 mm and a total absence of rain in the week prior to the sampling. This represents 49% of the 30 years average rainfall for that month in this area. We could, therefore, assume that a decrease in soil moisture content could have forced these worms to burrow into deeper layers and perhaps to feed on older C sources.

Full interpretation of these results requires more information about C assimilation in other earthworm species sampled at different seasons and from a greater variety of soils and climates to see how the ecological groupings are affected by environmental changes. Recent climate change research has put much emphasis on the biological regulation of C flow in terrestrial ecosystems as soils contain enough carbon to increase atmospheric CO_2 concentrations three fold (RCEP, 1996). Functional classifications of soil fauna allow a better understanding of the role of soil biota in ecosystem processes. In this study, the use of ^{14}C to investigate C assimilation by earthworms has provided new insights into the feeding behaviour of an important faunal component of the soil system and facilitates our understanding of the role that it plays in carbon turnover. A better knowledge of belowground food webs is essential to predict future responses to global climate change.

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