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Short communication

Use of ^{14}C carbon dating to determine feeding behaviour of enchytraeids

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Abstract

There is a lack of information about the trophic position of enchytraeid species, and it is unknown whether their vertical distribution in the soil corresponds to different feeding strategies. This study, for the first time, employs radiocarbon techniques to investigate the in situ feeding behaviour of enchytraeids. Soil cores including the associated vegetation were taken from a cambic stagnohumic gley near the summit of Great Dun Fell (845 m) in 1996. Since enchytraeid populations are known to be affected by changes in temperature and moisture regimes in upland soils, additional sampling was carried out at the soil warming experiment at the same site. Soil cores were also taken from a blanket bog on Hard Hill (560 m) at Moor House National Nature Reserve in 1998. All cores were sliced in the field into five layers, each of 2 cm, to a total depth of 10 cm. A subsample of each layer was used for animal extraction, and a parallel soil sample was used for ^{14}C isotope analysis. Results indicate that enchytraeids assimilate carbon components which are predominantly of material that is ca. 5–10 years old. The vertical movements of the worms due to changing abiotic factors do not affect this as they show similar values at all depths. However, in response to warming, they could have changed their C source as a lower ^{14}C enrichment with depth was observed in the heated samples.

Keywords: Enchytraeids; ^{14}C-bomb; Carbon; Climate change

Enchytraeids are frequently the most dominant faunal component of moorland ecosystems and may comprise up to ca. 70% of the total above- and below-ground animal biomass in specific systems (Cragg, 1961). Populations are usually dominated by *Cognettia sphagnetorum* which is often concentrated in the upper layers of the soil with, typically, ca. 80% of the population being found in the top 3 cm; this species may also constitute up to 95% of the total enchytraeid biomass (Coulson and Whittaker, 1978). Climate change studies have demonstrated the ability of this species to move down through the soil in response to adverse environmental conditions (Briones et al., 1997), with important implications for nutrient cycling (Briones et al., 1998a,b). This vertical movement of the animals to avoid warmer temperatures can only be a short-term strategy unless the food sources at depth can maintain or increase population numbers. If suitable food at depth becomes scarce, or has low quality, downward migration will only provide a short-term survival mechanism. In order to assess the specific role of these organisms in the decomposition of organic matter it is necessary to get a better understanding of their feeding preferences in nature (Didden, 1993).

Several studies have shown some evidence of enchytraeids consuming mineral particles (e.g. Dunger, 1983), fungi (Dash et al., 1981), bacteria (e.g. Rashed et al., 1992; Krištůfek et al., 1995), oats and yeasts (e.g. Mellin, unpublished data, Borckmeyer et al., 1990), algae (e.g. Shtina et al., 1981) and even dead bodies of lumbricids and arthropods (Mellin, unpublished data). However, there is a lack of information about the existence of trophic groups within the enchytraeid communities, and whether their vertical distribution in the soil corresponds to different feeding strategies.

^{14}C dating of soil organic matter has proved to be a useful technique in determining organic matter turnover rates (e.g. Ladyman and Harkness, 1980; Stout and Goh, 1980; O'Brien, 1984) and to calculate mean residence times of organic matter (Harkness et al., 1986) and ecosystem C budgets (Harrison and Harkness, 1993; Harrison et al., 1990, 2000). Nuclear weapons testing during the period from 1954 to 1962 resulted in a massive input of ^{14}C into the earth's atmosphere and this transient enrichment has been used as a global-scale tracer for organic matter studies (Jenkinson, 1963). In this study we have explored the possibility of using radiocarbon techniques to investigate the in

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situ feeding behaviour of small soil organisms, such as enchytraeids, for the first time.

Soil cores with the associated vegetation (*Juncus squarrosus* L. with *Festuca ovina* L., *Deschampsia flexuosa* (L.) Trin. and *Polytrichum commune* L.) were taken from a cambic stagnohumic gley near the summit of Great Dun Fell (845 m) in 1996 (within the Moor House National Nature Reserve; Grid Ref. NY710322). Since enchytraeid populations are known to be affected by changes in temperature and moisture regimes in upland soils (Briones et al., 1997), additional sampling was carried out at the soil warming experiment at the same site. This experimental design involved the use of a novel warming technique, which is able to provide a uniform heating of the vegetation/soil interface (for full details see Ineson et al., 1998). Soil and enchytraeid samples were taken from both the heated and unheated cores.

In 1998, soil cores were also taken from a blanket bog with peat on Hard Hill (560 m) at Moor House National Nature Reserve (Grid Ref. NY751333) where the vegetation is dominated by *Eriophorum vaginatum* L., *Calluna vulgaris* L. and *Sphagnum* moss.

All cores were sliced in the field into five layers, each of 2 cm, to a total depth of 10 cm and a subsample of each layer was used for animal extraction using a modified wet funnel method (O'Connor, 1955) using deionised distilled water. A parallel subsample was used for ^{14}C isotope analysis. Stones (>2 mm) were removed by sieving and living plant material and coarse roots were removed prior to drying (Harrison et al., 2000). Enchytraeids were separated according to species for the Great Dun Fell samples, whereas those from Moor House were bulked into single samples because of low population densities. Samples of soils and animals for ^{14}C analyses were, respectively, oven-dried at 65 °C for 24 h or freeze-dried. Soil and worm samples were analysed for ^{14}C concentration by accelerator mass spectrometry (AMS) (Pilcher, 1991). No pre-treatment of the samples was required and their preparation prior to isotopic analysis was carried out at the NERC Radiocarbon Laboratory (East Kilbride, Glasgow). 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 950 °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 University of Arizona NSF Accelerator Facility.

Ages were calculated as conventional radiocarbon years (before AD 1950). ^{14}C concentrations were expressed as percentage modern after normalisation of the measured radiometric enrichment of each sample relative to $\delta^{13}C_{PDB} = -25‰$ (Stuiver and Polach, 1977; Donahue et al., 1990). Final values are expressed as absolute percentage of modern ^{14}C calculated by mathematical adjustment to account for ongoing radioactive decay of the international ^{14}C reference standard (oxalic acid) since AD 1950.

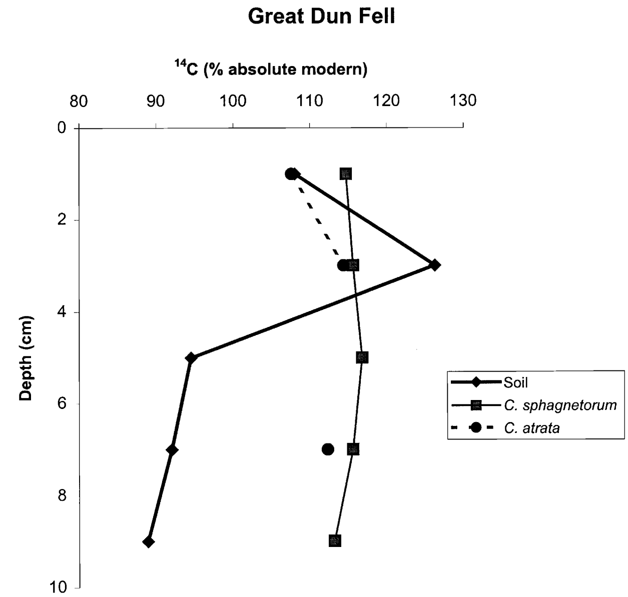

Fig. 1. Vertical distribution of ^{14}C enrichment in the soils and two species of enchytraeids from Great Dun Fell.

At Great Dun Fell a ^{14}C enrichment maximum had been incorporated to a depth of only 3 cm and rapidly decreased with each successive cm layer (Fig. 1). Little variation was observed in the enchytraeids ^{14}C values irrespective of where the animals were living in the soil profile, strongly suggesting that these animals assimilate C from the same sources. However, values for *C. sphagnetorum* were higher (approximately, 116 absolute percent modern carbon) than

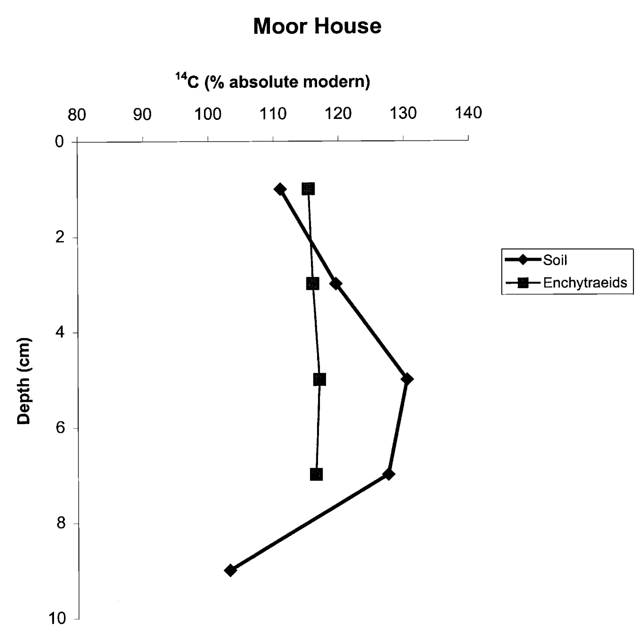

Fig. 2. Vertical distribution of ^{14}C enrichment in the soils and enchytraeids from Moor House.

the surface soil sample (108% absolute modern C). In contrast, surface *Cernosvitoviella atrata* showed an absolute percent modern carbon value similar to the surface soil sample, suggesting that it has assimilated more recent material.

Soils at Moor House show a deeper incorporation of the bomb C-14 peak (>7 cm), suggesting a more rapid accumulation of soil organic matter at this site (Fig. 2). Enchytraeid ^{14}C values differed little with soil depth, suggesting again that they have been assimilating the same C source. Additionally, animal values are a bit higher (approximately, 116 absolute percent modern carbon) than the surface soil sample (110% absolute modern C).

C. sphagnetorum extracted from the heating/unheating experiment showed similar values to the other sites in the unheated treatment and therefore it assimilated similar aged material which appear to have been fixed ca. 5–10 years before sampling. The surface sample of the heated treatment had similar C-14 value to the unheated samples, suggesting no treatment effect; however, the heated sample at 2–4 cm has a lower ^{14}C enrichment of 110.8 pmC, suggesting that different aged C has been assimilated.

From the above it is concluded that there was a different incorporation of ^{14}C into the soil organic matter between the two sites, with lowest ^{14}C enrichment values at the higher site. Little variation was observed in enchytraeids ^{14}C values with depth, suggesting that they are feeding on the same sources. Unlike *C. atrata* at the soil surface, *C. sphagnetorum* had higher absolute percent modern C values than the surface soil sample at both sites. ^{14}C values of vegetation reference material collected in 1991 and 1998 by the Radiocarbon Lab (East Kilbride, Glasgow) show an absolute percent modern C values of 116 and 110%, respectively. Accordingly, the enchytraeids at both sites appear to have assimilated carbon which is on average 5–10 years old (i.e. removed from the atmosphere in 1991).

A number of studies, including cultures (Gotthold et al., 1967; Gotthold and Koch, 1974; Palka and Spaul, 1970), gut content analyses (O'Connor, 1967; Dash and Cragg, 1972; Dash et al., 1980; Toutain et al., 1982; Ponge, 1991) and enzymatic analyses (Nielsen, 1962; Dash et al., 1981) have been performed on different enchytraeid species leading to the conclusion that microorganisms, and more especially fungal mycelia (Dash and Cragg, 1972), play a crucial role in the nutrition of these animals.

From axenic cultures of *C. sphagnetorum* Latter (1977) found that the growth of this species was similar to, or even better than, that for non-sterile worms on the same food, demonstrating that microbivory might not be the main feeding strategy in enchytraeids. Standen and Latter (1977) performed gut content analyses of *C. sphagnetorum* from three different habitats in a blanket bog (*Calluna*, *Eriophorum* and *Sphagnum*) finding that worms ingested mixed decomposing litter as available in the habitat. They also observed that the ingested litter included cellulosic and humified material containing fungal hyphae, but the results did not indicate selection of fungi from the food available to worms or any positive relationship between numbers of worms in a microhabitat and the quantity of active microorganisms that it contains. Similarly, Springett and Latter (1977) carried out field and laboratory tests to investigate the importance of microorganisms in the food of enchytraeids finding that the study did not provide a firm evidence that microorganisms themselves form any part of the natural diet of moorland Enchytraeidae, but the use of soluble nutrients is a possibility. On the other hand, Latter and Howson (1978) performed culture studies using litters of different ages to examine the feeding of *C. sphagnetorum*, finding that this species basically feeds on organic matter, mainly older litter of *Calluna* and *Eriophorum* plants. Fungi or bacteria as their sole source of food showed very poor growth and therefore the worm is considered as a primary decomposer. Furthermore, ultrastructural studies by Toutain et al. (1982) showed that enchytraeids are saprovores consuming plant debris.

The most obvious interpretation of our results from ^{14}C analyses is that C assimilation by these organisms appears to consist predominantly of material that is ca. 5–10 years old, and that the vertical movements of the worms due to changing abiotic factors are not affecting this as they show similar values at all depths.

Additionally results showed that the age of their C source changed in response to heating and therefore continued warmer conditions could have altered the source of C assimilation in these organisms.

Full interpretation of the radiocarbon data requires more detail on the mean C age of the organic inputs. However, this first use of 'natural' ^{14}C in the study of C assimilation by enchytraeids has provided new insights into the feeding behaviour of an important faunal component of the soil system and should be extended to other soil animal groups to get a better understanding of soil ecosystem functioning.

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References

Borckmeyer, V., Schmid, R., Westheide, W., 1990. Quantitative investigations of the food of two terrestrial enchytraeid species (Oligochaeta). Pedobiologia 34, 151–156.

Boutton, T.W., Wong, W.W., Hachley, D.L., Lee, L.S., Cabrera, M.P., Klein, P.D., 1983. Comparison of quartz and pyrex tubes for combustion of organic samples for stable isotopes. Analytical Chemistry 55, 1832–1833.

Briones, M.J.I., Ineson, P., Piearce, T.G., 1997. Effects of climate change on soil fauna; responses of enchytraeids, Diptera larvae and tardigrades in a transplant experiment. Applied Soil Ecology 6, 117–134.

Briones, M.J.I., Carreira, J., Ineson, P., 1998a. *Cognettia sphagnetorum* (Enchytraeidae) and nutrient cycling in organic soils: a microcosm experiment. Applied Soil Ecology 9, 289–294.

Briones, M.J.I., Ineson, P., Poskitt, J., 1998b. Climate change and *Cognettia sphagnetorum*: effects on carbon dynamics in organic soils. Functional Ecology 12, 528–535.

Coulson, J.C., Whittaker, J.B., 1978. Ecology of moorland animals. In: Heal, O.W., Perkins, D.F. (Eds.). Production Ecology of British Moors and Montane Grasslands. Springer, Berlin, pp. 52–93.

Cragg, J.B., 1961. Some aspects of the ecology of moorland animals. Journal of Ecology 49, 477–506.

Dash, M.C., Cragg, J.B., 1972. Selection of microfungi by Enchytraeidae (Oligochaeta) and other members of the soil fauna. Pedobiologia 12, 282–286.

Dash, M.C., Nanda, B., Behera, N., 1980. Fungal feeding by Enchytraeidae (Oligochaeta) in a tropical woodland in Orissa, India. Oikos 34, 202–205.

Dash, M.C., Nanda, B., Mishra, P.C., 1981. Digestive enzymes in three species of Enchytraeidae (Oligochaeta). Oikos 36, 316–318.

Didden, W.A.M., 1993. Ecology of terrestrial Enchytraeidae. Pedobiologia 37, 2–29.

Donahue, D.J., Linick, T.W., Jull, A.J.T., 1990. Isotope ratio and background corrections for accelerator mass spectrometry radiocarbon measurements. Radiocarbon 32, 135–142.

Dunger, W., 1983. Tiere im Boden. A. Ziemsen, Lutherstadt Wittenberg pp. 280.

Gotthold, M.L., Koch, J., 1974. Further development of an artificial medium for the axenically cultured annelid *Enchytraeus fragmentosus*. Comparative Biochemistry and Biophysics 48B, 307–314.

Gotthold, M.L., Brody, B., Stokstad, E., 1967. Axenic cultivation of the microannelid *Enchytraeus fragmentosus* (Bell, 1959). Comparative Biochemistry and Physiology 21, 75–81.

Harkness, D.D., Harrison, A.F., Bacon, P.J., 1986. Temporal distribution of bomb ^{14}C in a forest soil. Radiocarbon 28, 328–337.

Harrison, A.F., Harkness, D.D., 1993. The potential for estimating carbon fluxes in forest soils using ^{14}C techniques. New Zealand Journal of Forest Science 23, 367–379.

Harrison, A.F., Harkness, D.D., Bacon, P.J., 1990. The use of bomb-^{14}C for studing organic matter and N and P dynamics in a woodland soil. In: Harrison, A.F., Ineson, P., Heal, O.W. (Eds.). Nutrient cycling in terrestrial ecosystems: field methods, application and interpretation. Elsevier, London, pp. 246–258.

Harrison, A.F., Harkness, D.D., Rowland, J.S., Garnett, J.S., Bacon, P.J., 2000. Annual carbon and nitrogen fluxes in soils along the European forest transect, determined using ^{14}C-bomb. In: Schulze, E.D. (Ed.). Carbon and Nitrogen Cycling in European Forest Ecosystems. Springer, Berlin, pp. 237–254.

Ineson, P., Benham, D.G., Poskitt, J., Harrison, A.F., Taylor, K., Woof, C., 1998. Effects of climate change on nitrogen dynamics in upland soils. 2. A soil warming study. Global Change Biology 4, 153–161.

Jenkinson, D.S., 1963. The turnover of organic matter in soil. In: The Use of Isotopes in Soil Organic Matter Studies. FAO/IAEA Technical Meeting Report (Volkenrode). Pergamon Press, Oxford, pp. 187–198.

Krištůfek, V., Hallmann, M., Westheide, W., Schrempf, H., 1995. Selection of various *Streptomyces* species by *Enchytraeus crypticus* (Oligochaeta). Pedobiologia 39, 547–554.

Ladyman, S.J., Harkness, D.D., 1980. Carbon isotope measurement as an index of soil development. In: Stuiver, M., Kra, S.A. (Eds.), Proceedings of the 10th International Radiocarbon Conference. Radiocarbon 22, 885–891.

Latter, P.M., 1977. Axenic cultivation of an enchytraeid worm, *Cognettia sphagnetorum*. Oecologia 31, 251–254.

Latter, P.M., Howson, G., 1978. Studies on the microfauna of blanket bog with particular reference to Enchytraeidae. II. Growth and survival of *Cognettia sphagnetorum* on various substrates. Journal of Animal Ecology 47, 425–448.

Nielsen, C.O., 1962. Carbohydrases in soil and litter invertebrates. Oikos 13, 200–215.

O'Brien, B.J., 1984. Soil organic carbon fluxes and turnover rates estimated from radiocarbon enrichments. Soil Biology and Biochemistry 16, 115–120.

O'Connor, F.B., 1955. Extraction of enchytraeid worms from a coniferous forest soil. Nature 175, 815–816.

O'Connor, F.B., 1967. The Enchytraeidae. In: Burges, A., Raw, F. (Eds.). Soil Biology. Academic Press, London, pp. 213–257.

Palka, J., Spaul, E.A., 1970. Studies of feeding and digestion in the enchytraeid worm *Lumbricillus lineatus* Müll. in relation to its activity in sewage bacteria beds. Proceedings of the Leeds Philosophical and Literary Society, vol. 10, pp. 45–59.

Pilcher, J., 1991. Radiocarbon dating. In: Smart, P.L., Francis, P.D. (Eds.), Quaternary Dating Methods—A User's Guide. Technical Guide No. 4. Quaternary Research Association, London, pp. 16–36.

Ponge, J.F., 1991. Food resources and diets of soil animals in a small area of Scots pine litter. Geoderma 49, 33–62.

Rashed, H.A., Szabó, I.M., Dózsa-Farkas, K., 1992. On the composition of the instestinal microbiota of *Fridericia hegemon* (Enchytraeidae). Soil Biology and Biochemistry 24, 1291–1294.

Shtina, E.A., Kozlovskaya, L.S., Nekrasova, K.A., 1981. Relations of soil oligochaetes and algae. Soviet Journal of Ecology 12, 44–48.

Slota, P.J., Jull, A.J.T., Linick, T.W., Toolin, L.J., 1987. Preparation of small samples for ^{14}C accelerator targets by catalytic reduction of CO. Radiocarbon 29, 303–306.

Springett, J.A., Latter, P.M., 1977. Studies on the microfauna of blanket bog with particular reference to Enchytraeidae. I. Field and laboratory tests of micro-organisms as food. Journal of Animal Ecology 46, 959–974.

Standen, V., Latter, P.M., 1977. Distribution of a population of *Cognettia sphagnetorum* (Enchytraeidae) in relation to microhabitats in a blanket bog. Journal of Animal Ecology 46, 213–219.

Stout, J.D., Goh, K.M., 1980. The use of radiocarbon to measure the effects of earthworms on soil development. In: Stuiver, M., Kra, S.A. (Eds.), Proceedings of the 10th International Radiocarbon Conference. Radiocarbon 22, 892–896.

Stuiver, M., Polach, H.A., 1977. Discussion: reporting of ^{14}C data. Radiocarbon 19, 355–363.

Toutain, F., Villemin, G., Albrecht, A., Reisinger, O., 1982. Etude ultrastructurale des processus de biodégradation. II. Modèle enchytraeides—litière de feuilles. Pedobiologia 23, 145–156.