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SHORT COMMUNICATION

DO EARTHWORMS EAT LIVING ROOTS?

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The importance of earthworms for increasing plant productivity is well known (Stockdill and Cossens, 1969; Edwards and Lofty, 1972, 1978). However, we have shown (Hameed *et al.*, 1992) that in microcosms, *Lumbricus terrestris* could enhance N accumulation in plants while decreasing plant production, particularly in the root system. To explain this observation, one of the suggested hypotheses was that earthworms could eat living roots. To date, little attention has been paid to this problem because organic debris and soil organic matter are known to constitute the main diet of earthworms. In the past, "worms" were also described as root feeders. But, in the last century, the recognition of *Annelida* and then of earthworms, led to confer on this terrestrial group a feeding based only on dead organic material. In that way, Darwin (1881) fed earthworms with dead animals and plant remains (but no roots) and Hensen (1877, 1882, 1892) established the importance of earthworms as a major influence on root growth but nothing was suggested about feeding on living roots. Later, Bouché and Kretschmar (1974) and Ferrière (1980) observed roots in the gut contents of some earthworms but considered them to be dead. In contrast, Gerard (1963) supports the view that some earthworm species may be living root feeders but with no evidence. Thus far, however, no one has observed that living roots could be a food source for earthworms. If living roots were found in the gut contents of earthworms, this would provide conclusive evidence that the animal had ingested them. The first step is to prove that the ingested roots were really living. To test the hypothesis that living roots could be a food source for earthworms, we have carried out a short-pulse labelling experiment.

The principle of the experiment is as follows: if $^{14}CO_2$ is photosynthesized by plants according to the short-pulse labelling technique (McDougall and Rovira, 1965), the new living fine roots developed during that time (24 h) will be ^{14}C labelled. After the plants have been placed in a non-labelled atmosphere, labelled plant fragments found in the gut of earthworms will come exclusively from these living roots. However, it is difficult to separate plant fragments for gut contents and, for that reason, we measured ^{14}C and total C (^{t}C) in the gut contents as a whole. The radioactivity measured in the gut could also come from (1) microorganisms labelled after consumption of ^{14}C exudates and (2) ^{14}C microbial metabolites. However, the time between labelling and sampling of the gut contents is too short to allow any significant turnover of C in the microflora.

Adult earthworms (*Lumbricus terrestris* L.) were collected in the field by the formalin method (Raw, 1959), washed with H_2O, placed into the same soil at 14°C and 0.098 MPa and fed for 3 or 4 weeks. By then, the earthworms were fully active.

For the experiment, soil was collected from the upper 20 cm of the A1 horizon of a fersialitic calcic soil developed under humic mediterranean climatic conditions (clay = 28%; silt = 43%; sand = 29%; C = 1.5%; N = 0.12%; pH H_2O = 7.6; $CaCO_3$ 2.3%). The air-dry soil was sieved (2 mm), homogenized, moistened to 80% WHC, put in 9 plastic pots (850 g air-dry wt equivalent each) and sown with rye-grass (*Lolium perenne* L.; 15 seedlings per pot). The pots were then placed in a greenhouse with a 16 h-day at 22°C and an 8 h-night at 16°C. Soil moisture was maintained at 80% WHC during the experiment.

After 6 weeks, two earthworms were added to each pot. Two weeks later, ^{14}C plants were labelled for 24 h according to the pulse-labelling technique (McDougall and Rovira, 1965) in a chamber derived from that described by Warembourg *et al.* (1982). After this short labelling period, pots were again placed in the greenhouse for 24 h, in a non-labelled atmosphere. The repartition of ^{14}C in soil, roots, surface casts, earthworm bodys and earthworm gut contents was then determined, as follows:

(1) The plants were harvested, the root tips were cut (0.5 cm length), carefully washed and oven-dried at 40°C.
(2) The soil of each pot was cleared of remaining roots by sieving and hand extraction, and oven-dried at 70°C.
(3) The earthworms were dipped in boiling water for 30 s and then dissected. Gut content was collected by several washings with distilled H_2O, evaporated and oven-dried at 40°C. Earthworm bodies were also oven-dried at 40°C.
(4) The surface casts produced during the 24 h following the ^{14}C short labelling were collected and oven-dried at 70°C.

After drying, root tips, earthworms, gut contents and surface casts were ground in a mortar for ^{t}C and ^{14}C analysis. ^{t}C was determined by dry combustion at 900°C with a Carmhograph 12-A and ^{14}C by scintillation counting after collecting the CO_2 in ethanolamine and 2-methoxyethanol (w/v = 1/3) (Bottner and Warembourg, 1976). After dissection of the earthworms, some plant debris were identifiable in gut contents, but because of their small quantities, the determination of ^{t}C and ^{14}C was carried out on the gut contents as a whole.

Specific activities occurred in the following order: roots > gut contents ≫ soil > casts > earthworm tissues (Table 1). These results confirm that labelled C was ingested by earthworms. ^{t}C was relatively close irrespective of gut content (mean 90.19 ± 5.45 mg g^{-1} dry gut content; Table 2).The quantity of $^{*}C$ originating from roots and found in the gut contents varied, however, among pots and even in the same pot (pots 6 and 8). Four earthworms showed no labelling in their gut content (GC62, GC71, GC72, GC82, GC91, GC92) indicating no root feeding. In pots 6 and 8, it is noteworthy that the gut content of only one earthworm was labelled showing by that the variability

Table 1. Distribution of ^{14}C, ^{t}C, [^{14}C]specific activity and *C in soils (mean ± SE, $P < 0.05$; $n = 9$), fine roots (mean ± SE, $P < 0.05$; $n = 9$), earthworms (mean ± SE, $P < 0.05$; $n = 18$), gut contents (mean ± SE, $P < 0.05$; $n = 10$) and casts (mean ± SE, $P < 0.05$; $n = 9$) after a 24 h short pulse labelling

	^{14}C (Bq g^{-1}) (1)	Total C (mg g^{-1}) (2)	[^{14}C]specific activity (3)	*C (mg g^{-1}) (4)
Soil	30 ± 4	16.22 ± 0.16	1.92 ± 0.25	7.00 ± 0.01
Roots	102687 ± 18264	347.92 ± 16.63	295.02 ± 49.67	
Earthworms	36 ± 10	431.21 ± 18.18	0.08 ± 0.02	0.12 ± 0.03
Gut content	4639 ± 1406	90.19 ± 5.45	52.00 ± 15.00	15.70 ± 4.80
Casts	99 ± 30	74.43 ± 2.03	1.33 ± 0.80	0.34 ± 0.20

[^{14}C]specific activity = $^{14}C/^{t}C$ is expressed in Bq $mg^{-1}C$.
*C represents the labelled C originating from roots. It corresponds to the following ratio: ^{14}C (from samples): root [^{14}C]specific activity (=295.02).
Columns (1), (2) and (4) are expressed by g of sample.

of feeding behaviour. *C from the labelled gut contents varied from 7.34 to 23.84 mg *C g^{-1} dry gut content (mean 15.70 ± 4.80 mg *C g^{-1} dry gut content). This labelling apparently came not only from browsed living roots and rhizosphere microorganisms living on them, but also from microbial metabolites and labelled plant residues released into the soil. The rhizosphere microflora that was in contact with roots was necessarily ingested when earthworms consumed roots.

Plant shot-term labelling is mostly observed on growing root parts. *Lumbricus terrestris* invariably consume a mixture of soil including the resident organic matter and plant tissues usually considered as dead. The observed specific activity well reflected an intermediate value between specific activity of soil and roots. It was noticeable that some earthworms fed on soil with a lower specific activity than that of soil as a whole (Table 1). Then some earthworms selected old and unlabelled organic matter while most of them added to their diets a significant fraction of (1) young roots surrounded with a rhizosphere microflora and (2) recent young root residues. In such a short time span, most of the ^{14}C ingested came from living roots.

Table 2. Distribution of ^{14}C, ^{t}C, [^{14}C]specific activity and *C in gut contents (mean ± SE, $P < 0.05$; $n = 10$) after a 24 h short pulse labelling

	^{14}C (Bq g^{-1}) (1)	Total C (mg g^{-1}) (2)	[^{14}C]specific activity (3)	*C (mg g^{-1}) (4)
GC11	3865	85.81	45.03	13.10
GC12	4739	86.38	54.86	16.06
GC21	ND	ND	ND	ND
GC22	ND	ND	ND	ND
GC31	4852	83.70	57.97	16.45
GC32	4925	83.37	59.07	16.69
GC41	3737	85.78	43.56	12.67
GC42	2165	85.30	25.38	7.34
GC51	5561	96.62	57.55	18.85
GC52	6084	96.58	62.99	20.62
GC61	3430	99.01	34.64	11.63
GC62	224	100.51	2.33	0.76
GC71	70	89.27	0.78	0.24
GC72	110	87.76	1.25	0.37
GC81	7033	91.87	76.55	23.84
GC82	98	91.88	1.07	0.33
GC91	49	89.62	0.55	0.17
GC92	73	89.53	0.82	0.25
Mean ± SE	4639	90.19	52.00	15.70
	1406	5.45	15.00	4.80

Samples GC62 (gut content, pot No. 6, 2nd earthworm), GC71, GC72, GC82, GC91 and GC92 were not included in the means. Gut contents from 2nd pot were lost.
[^{14}C]specific activity = $^{14}C:^{t}C$ is expressed in Bq $mg^{-1}C$.
*C represents the labelled C originating from roots. It corresponds to the following ratio: ^{14}C (from guts): root [^{14}C]specific activity (=295.02).
Columns (1), (2) and (4) are expressed by g of sample.
ND = non determined.

Because earthworms consume dead organic matter litter and plant debris collected on the surface of soil, the general opinion is that the diet of earthworms is exclusively composed of dead material. Our experiment showed that, at least under our experimental conditions, some earthworms tested consumed appreciable amounts of living roots. Food consumption is related to palatability (Cooke and Luxton, 1980; Harstentein, 1982; Cortez and Hameed, 1988; Cortez *et al.*, 1989) and young roots seemed palatable for *Lumbricus terrestris*. This herbivory could induce a stimulation of plant growth as observed under managed grazing of cattle or could depress plant growth under overgrazing. The observation of decreasing plant production (Hameed *et al.*, 1992) could be induced by an excess of earthworms in the microcosms. In the field, a substantial root system often develops in earthworm burrows. Thus, it would be interesting to verify if root consumption is similar under field conditions and in microcosms. If so, it would be necessary to quantify this process in the field to estimate its effect both on plants and earthworms.

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