Lumbricus Terrestris-Mediated Carbon Transport Into Soil and Soil Microbial Communities

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Study Rationale

•The burrowing activity of anecic earthworms can facilitate the incorporation of fresh residues into soil and creates distinct habitats (casts, middens and burrows) that support active microbial communities.

•Surprisingly, we know very little about the quantity of C incorporated or the composition and diversity of microbial communities in earthworm "hotspots", including the drilosphere, or lining of earthworm burrows.

Objectives

•Apply natural abundance stable isotope probing (SIP) to quantify the effect of *Lumbricus terrestris* on C incorporation into soil, microbial community structure, and assimilation of plant C into distinct populations of drilosphere microbial communities.

Methods

•The study began in April 2009, in a set-aside pasture located on the UCD Research Farm, Lyons Estate, Celbridge, Co. Kildare. **The site was sown to perennial ryegrass (C_3 species) in Fall 2001, and prior to this, the area was cropped to maize (C_4 species) over a period of 4-5 years.**

•In each of five 1-m ^{2} replicate plots, one occupied burrow of *L. terrestris* was identified and excavated with a 15-cm diameter corer or shovel (Figs. 1-2). The soil block was divided into two depth increments, 0-5 cm and 5-15 cm. **Drilosphere soil was collected from 5-15 cm depth increment** using a spatula to scrape away soil within 2-5 mm of the burrow lining (Fig. 3). **Non-drilosphere (bulk) soil was collected at least 2 cm away from the burrow**, from both the 0-5 and 5-15 cm increments.

•Aboveground plant biomass was harvested and separated into grass, herb and legume pools. Oven-dried plants were weighed, ground and analyzed for δ^{13} by GC isotopic ratio mass spectrometry (IRMS).

•Soils were freeze-dried, ground and analyzed for δ^{13} by IRMS. Microbial phospholipid fatty acids (PLFAs) were extracted and the δ^{13}C value was determined for individual PLFAs by GC-combustion-IRMS (UC Davis Stable Isotope Facility in Davis, CA).

Fig. 1. Opening of a *Lumbricus terrestris* burrow

Fig. 2. *L. terrestris* emerging from burrow after mustard oil application.

Fig. 3. Excavation of drilosphere soil from *L. terrestris* burrow.

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Study Progress and Results to Date

Drilosphere Soil Carbon

•*L. terrestris* and their burrows facilitated the incorporation of C_3 plant carbon at depth in soil.

•Except for one burrow, drilosphere soil δ^{13}C was more negative than that of non-drilosphere soil at the 5-15 cm depth (Fig. 4).

•The proportion of drilosphere soil C originating from C_3 plants ranged from 13 to 48 % (n=4), according to a simple two-source mixing model using δ^{13}C values from plants, drilosphere soil, and 5-15 cm bulk soil.

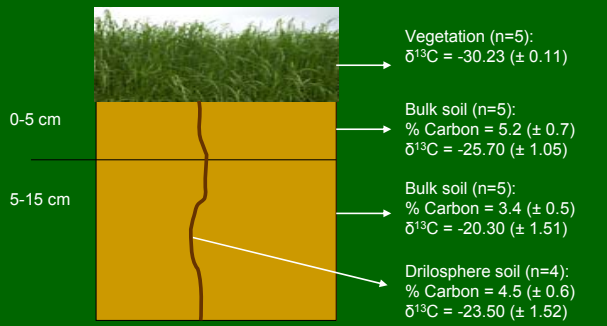

Fig. 4. Schematic of soil block (0-15 cm depth) with soil carbon and δ^{13}C results. Values are means ± 1 standard deviation.

Drilosphere Microbial Communities

•*L. terrestris* burrows supported distinct microbial communities that were large in biomass but slightly less diverse and PLFA-rich, relative to 5-15 cm bulk soil.

•Microbial PLFA biomass and diversity was similar between drilosphere soil (5-15 cm depth) and 0-5 cm bulk soil (Table 1), according to ANOVA tests ($p < 0.05$).

•PLFA richness was lowest in the drilosphere (Table 1). PLFAs absent from all or most drilosphere samples were branched PLFAs (15:1, 16:1, and 19:1), 17:1 *iso*, and 17:1.

•Principal components analysis of PLFAs showed that 4 of 5 drilosphere communities (5-15 cm depth) were intermediate between 0-5 cm and 5-15 cm bulk soil communities along PC1, and were distinct from bulk soil communities along PC 2 (Fig. 5).

•Drilosphere microbial communities were enriched in certain bacterial (16:0 iso and 17:0 cyclo), fungal/plant (18:2 ω6c), and protozoa (20:4 ω6c) PLFAs compared to bulk soil communities (Table 2).

•The drilosphere sample whose δ^{13}C value (-20.62) was similar to that of bulk soil (-20.84) had a unique microbial community structure, identified as the outlier on Fig. 5.

•The proportion of carbon from C3 plants assimilated into drilosphere microbial PLFAs has yet to be determined.

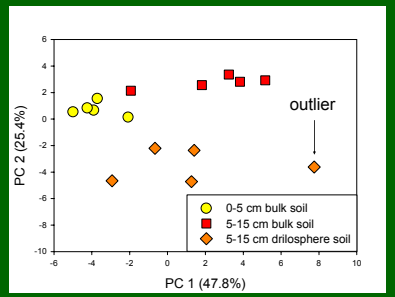

Fig. 5. Principal components analysis of microbial PLFAs. The percent variance explained by each principal component (PC) is shown in parentheses.

Table 1. PLFA concentration, richness and Simpson's diversity index in bulk and drilosphere soils.

Soil	µg PLFA-C g^{-1} soil	PLFA richness	PLFA Diversity
0-5 cm bulk	27.0a (± 5.4)	28.8a (± 0.4)	0.937b (± 0.002)
5-15 cm bulk	11.2b (± 3.0)	27.2b (± 1.0)	0.942a (± 0.002)
5-15 cm drilosphere	18.9a (± 5.3)	24.6c (± 1.3)	0.937b (±0.002)

Table 2. PLFAs with the greatest eigenvector loadings on each principal component (PC) of the PCA.

PC 1		PC 2	
PLFA	Loading	PLFA	Loading
15:0	0.24	15:0 *iso*	0.30
16:0 *12 Me*	0.24	15:1 *branched*	0.32
16:1 *2OH*	0.24	16:0 *10Me*	0.25
17:0 *iso*	0.26	19:1 *branched*	0.34
17:0 *anteiso*	0.23	14:0	-0.22
18:0 *10Me*	0.25	16:0	-0.26
19:0 *cyclo*	0.22	16:0 *iso*	-0.26
16:1 *ω7c/9c*	-0.23	17:0 *cyclo*	-0.19
16:1 *ω7t*	-0.22	18:2 *ω6c*	-0.19
17:1 *iso*	-0.22	20:4 *ω6c*	-0.25