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Original article

Use of stable isotopes (^{13}C) for studying the mobilisation of old soil organic carbon by endogeic earthworms (Lumbricidae)

Sven Marhan [a,b,*], Reinhard Langel [c], Ellen Kandeler [a], Stefan Scheu [b]

[a] *Institut für Bodenkunde und Standortslehre, Universität Hohenheim, Emil-Wolff-Strasse 27, 70599 Stuttgart, Germany*
[b] *Institut für Zoologie, Technische Universität Darmstadt, Schnittspahnstrasse 3, 64287 Darmstadt, Germany*
[c] *Kompetenzzentrum Stabile Isotope, Forschungszentrum Waldökosysteme, Georg-August Universität Göttingen, Büsgenweg 2, 37077 Göttingen, Germany*

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Abstract

Endogeic earthworms ingest large amounts of organic matter enclosed in mineral soil. Part of the soil organic matter is mobilised during the gut passage, but the overall effect of earthworms on the dynamics of soil organic carbon (SOC) is poorly understood because the origin and age of the mobilised SOC pool are unknown. To determine whether endogeic earthworms mobilise old SOC pools, we studied the effect of *Octolasion tyrtaeum* (Savigny) on ^{13}C signatures of CO_2 evolved from soil of a maize field incubated in microcosms with and without earthworms for 150 days. Cultivation on this field had changed from wheat (C_3 plant) to maize (C_4 plant) 23 years ago.

Earthworms generally decreased in body mass during the experiment but increased CO_2 production. Compared to the initial signature ($-22.8 \pm 0.1‰_{PDB}$), $\delta^{13}C$ signatures of *O. tyrtaeum* were more depleted in the wheat ($-23.6 \pm 0.3‰_{PDB}$) and more enriched in the maize soil ($-21.0 \pm 0.4‰_{PDB}$) at the end of the experiment. The $\delta^{13}CO_2$-C signatures in the wheat soil were not affected by earthworms, but earthworms decreased $\delta^{13}CO_2$-C values in the maize soil during the second half of the incubation period. This indicates enhanced mineralization of old wheat-derived carbon. The results suggest that lumbricid endogeic earthworms contribute to the mobilisation of old carbon pools in soils.

Keywords: *Octolasion tyrtaeum*; Mineralization; Soil organic matter; Carbon dioxide; C_3–C_4-plants

1. Introduction

Understanding soil organic matter dynamics requires knowing the parameters that affect the stability of soil organic carbon (SOC). SOC consists of different constituents that vary in mass, age and turnover rate. Plant residues and root exudates contribute to the labile fraction, whereas resistant humic substances may withstand decomposition for more than 1000 years [7]. SOC degradation and mineralization is mainly due to the activity of microorganisms. Soil animals, such as earthworms, alter the environmental conditions for microorganisms and therefore affect the decomposition of organic material [9,14,29]. In casts of endogeic earthworms from temperate climate regions microbial biomass was found to be reduced compared to bulk soil, while microbial activity (specific respiration) was found to be enhanced

* Corresponding author: Institut für Bodenkunde und Standortslehre, Universität Hohenheim, Emil-Wolff-Strasse 27, 70599 Stuttgart, Germany. Tel.: +49 711 4592 2614; fax: +49 711 4592 3117.
E-mail address: smarhan@gmx.de (S. Marhan).

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[27,28]. The mobilisation of nutrients, such as nitrogen and phosphorous, but also of easily available carbon sources, is responsible for the enhanced activity of soil microorganisms in earthworm casts [23].

The carbon assimilation efficiency of lumbricid endogeic earthworm species is low (1–2%), and they compensated for this by consuming large quantities of soil [5,22,26]. Little is known about which carbon pool endogeic earthworms actually mobilise and assimilate. The analysis of natural carbon stable isotope signatures ($\delta^{13}C$) might be a powerful tool to investigate the age of SOC pools mobilised by soil organisms [10,20]. Generally, the isotopic signatures of SOC reflect that of the local plant community [6,8]. Using soil from sites on which C_3 plants had been replaced by C_4 plants is an approach to determine the incorporation of plant-derived carbon into the SOC pool [2,3,11]. The $\delta^{13}C$ signatures of microorganisms (C_{mic}) and of the respired CO_2 reflect those of SOC. Analysing $\delta^{13}C$ signatures of CO_2 evolved from soils allows the actually mineralised carbon pool to be traced [20].

The objective of the present study was to determine the age of the SOC fraction which is mobilised by the lumbricid endogeic earthworm species *Octolasion tyrtaeum* (Savigny). Two soils of different cropping history were analysed. One originated from a field where cultivation shifted from wheat to maize 23 years ago; the other was taken from a field which had been planted with wheat since 1969. Analysing the $\delta^{13}C$ signature in CO_2 from treatments with and without *O. tyrtaeum* reveals whether this endogeic earthworm species mobilises the older C_3-derived SOC.

2. Material and methods

2.1. Soil sampling

Soil samples were taken from the long-term field experiment of the Landbauschule Rotthalmünster (Bavaria, Germany). The site is located 360 m above sea level, the mean annual precipitation is 886 mm and the mean annual temperature is 8.7 °C. The soil is a *stagnic Luvisol* (WAB) composed of approximately 11% sand, 73% silt and 16% clay [12]. Soil was taken from two fields: one continuously planted with wheat since 1969 and fertilised with NPK (wheat treatment), the other continuously planted with maize since 1979; the latter field had previously also been cultivated with wheat. The wheat and maize fields were fertilised with 171 and 180 kg N ha^{-1} yr^{-1}, respectively [12].

Soil samples were taken from the upper 30 cm (plough horizon) in September 2002, shortly after harvest (wheat field) and before harvest (maize field). At both fields, straw materials were left on the field and ploughed into the soil to a depth of 30 cm. The wheat field was tilled after harvest. Soil samples were taken from four locations per field and pooled (in total ~10 kg) (see also ref. [12]). All visible plant residues and stones were excluded by sieving (4 mm). The pH measured in 0.01 M $CaCl_2$ solution of the soil was 6.34 and 6.54 for the wheat and maize field, respectively. The soil organic carbon (C_{org}) and total nitrogen (N_{tot}) content of the wheat field was 12.8 mg C_{org} g^{-1} dry wt and 138 μg N_{tot} g^{-1} dry wt, respectively. Respective values of the maize field were 12.3 mg C_{org} g^{-1} dry wt and 134 μg N_{tot} g^{-1} dry wt.

The $\delta^{13}C$ signature of wheat plants was $-26.8 \pm 0.1‰_{PDB}$, that of maize plants $-12.7 \pm 0.2‰_{PDB}$ [12]. Soil ^{13}C-signatures were $-26.6 \pm 0.1‰_{PDB}$ for the wheat and $-21.3 \pm 0.3‰_{PDB}$ for the maize field, in the latter reflecting long-term maize cropping. About 35.7% of the total C_{org} in the Ap horizon of the maize field originated from maize carbon [12].

For the experiment, subadult specimens of the endogeic earthworm species *O. tyrtaeum* were extracted by hand from a 130-year-old beech forest near Göttingen ("Göttinger Wald") in October 2002.

2.2. The experiment

Soil samples were stored at 5 °C for one week, and for acclimation one further week at 20 °C before the start of the experiment. The experiment was set up in microcosms consisting of perspex tubes (height 150 mm, Ø 60 mm) fixed airtight on ceramic plates. The microcosms allow drainage of soil materials at semi-natural conditions by lowering the atmospheric pressure in a box below the ceramic plate. The microcosms were closed at the top by a lid bearing a small vessel attached to the underside. This vessel could be filled with alkali to absorb CO_2 evolved from the soil.

The microcosms were filled with fresh soil equivalent to 200 g dry wt. The following treatments were established: wheat soil without earthworms (Wheat Ctrl.), wheat soil with *O. tyrtaeum* (Wheat *O. tyr.*), maize soil without earthworms (Maize Ctrl.) and maize soil with *O. tyrtaeum* (Maize *O. tyr.*). For each treatment, 10 microcosms were set up. One *O. tyrtaeum* individual was added per microcosm. Before the earthworms were added they were placed on wet filter paper for three days to void their guts; the filter paper was changed twice. The mean initial body mass of the added *O. tyrtaeum* individuals was 255 (±50) mg fresh wt, but both smaller and larger specimens were used (Table 1).