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Short-term carbon mineralization from endogeic earthworm casts as influenced by properties of the ingested soil material

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ABSTRACT

Fresh casts of endogeic earthworms are considered a hotspot of microbial activity that exhibit greater carbon mineralization (C_{min}) than the bulk soil. While cast properties depend on the ingested soil material, little is known about how earthworm feeding behavior and digestive processes interact with those properties to determine the C_{min} in fresh casts egested by endogeic earthworms. Two laboratory experiments were designed to (i) assess the short-term changes in C_{min} of soil and fresh casts after the soil or soil physical size fractions passed through the gut of *Aporrectodea caliginosa*, a common endogeic species in temperate agroecosystems, and (ii) to determine whether these changes depended on initial properties of the ingested materials. In the first experiment, we determined how C_{min} was affected by gut passage (i.e., casts vs. bulk and surrounding soil) and soil type (three sandy-loam soils with variable content of light fraction organic matter (LF): the Courval, St. Amable and Chicot soil series). In the second experiment, we related C_{min} in casts to the interactive effect of the gut passage and soil fraction size (Courval soil series only). Six soil treatments were examined: whole soil and five-soil fraction size classes (2000–1000 μm, 1000–500 μm, 500–250 μm, 250–53 μm and < 53 μm). As hypothesized, the earthworm gut transit increased C_{min} in casts by two to three-fold relative to the bulk soil and surrounding soil, and the increase in cast C_{min} was soil- and soil fraction size-specific. The C_{min} in casts was significantly ($p < 0.05$) greater in the finest soil fraction (< 53 μm) and lowest in the intermediate fraction (500–250 μm) compared to the whole soil and other soil fractions. Additionally, the priming effect of earthworm ingestion and digestion processes, estimated by the normalized C_{min} for casts (which subtracts the baseline C_{min} flux from the bulk soil or soil fraction) was positively correlated ($p < 0.05$) with the C concentration of the LF in the ingested soil. This suggests that *A. caliginosa* derive their nutrition from the LF, and that the 500–250 μm fraction is the optimal size to support their nutritional requirements.

1. Introduction

Earthworms contribute to soil organic matter dynamics in agroecosystems by consuming an estimated 2 to 15 Mg ha^{-1} $year^{-1}$ of organic residues and procesings as much as 10% of the topsoil each year (Whalen and Parmelee, 2000). Once the organic materials and soil are ingested, they are mixed with intestinal mucus, and decomposed by enzymes originating from earthworms, ingested microorganisms and indigenous gut microflora (Brown et al., 2000). As soil passes through the earthworm gut, many changes occur in its chemical, physical, and biological properties until the undigested materials are deposited as casts. In the short term, fresh earthworm casts are known to be a hotspot of intensive microbial activity, accelerated decomposition, and thus enhanced carbon mineralization (C_{min}; Lavelle, 1988; Martin and Marinissen, 1993; Tiunov and Scheu, 2000). Although C_{min} declines with time as aging casts become drier and microbial activity slows (Marinissen and Dexter, 1990; Marinissen, 1994; Aira et al., 2010), the CO_2 efflux may be considerable in temperate agroecosystems where cast production is estimated between 36 and 108 Mg ha^{-1} $year^{-1}$ (Lavelle and Spain, 2001).

Of particular interest is the cast production of endogeic earthworms, which are numerically dominant in temperate agroecosystems (Whalen and Fox, 2007). Endogeic earthworms inhabit and derive their nutrition from the mineral soil horizon. They deposited 30–142 mg g^{-1} earthworm fresh weight d^{-1} of casts on the surface and within soil mesocosms incubated at 10 to 20 °C (Whalen et al., 2004). In general, fresh casts of endogeic earthworms contain more available nutrients (N, P, K and Ca) and support more microbial activity than the bulk soil

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(Marinissen, 1994; Bossuyt et al., 2004; Aira and Domínguez 2014). Consequently, a greater C_{min} rate was reported in fresh casts of endogeic earthworms than the bulk soil (Scheu, 1987; Aira et al., 2003). However, these characteristics of earthworm casts are likely to be species-specific and to depend as well on the initial properties of the ingested material. It is established that casts produced by different earthworm species have different properties, even when those species belong to the same ecological group and feed on the same material (Zhang et al., 2009; Bottinelli et al., 2010; Shan et al., 2010; Aira and Domínguez 2014; Clause et al., 2014; Chang et al., 2016). According to these authors, the observed differences in casts result from several factors: the food selected by earthworms, the digestive capability of earthworms and associated gut microbes, and the nature of the ingested material, which includes both organic residues and soil.

Endogeic earthworms are geophagous, meaning that they consume soil and derive their nutrition primarily from the soil organic matter (Edwards, 2004; Curry and Schmidt, 2007). Thus, casts of endogeic earthworms should be influenced by characteristics of the ingested soil. Although the physico-chemical properties of fresh casts from endogeic species are strongly related to the properties of the ingested soil (Zhang and Schrader, 1993; Schrader and Zhang, 1997; Zhang et al., 2009; Hedde et al., 2013; Clause et al., 2014; Wachendorf et al., 2014), the relationship between biological properties of fresh casts and initial soil properties is poorly defined. An indicator of microbial activity like C_{min} is useful to compare biological properties of fresh casts when endogeic earthworms consume soils with diverse textural properties, organic matter content and microbial community composition. The working hypotheses for the study are (i) fresh casts will have greater C_{min} than uningested soil, and (ii) the magnitude of the increase in C_{min} in casts versus uningested soil will be related to the amount of partially decomposed organic matter (i.e., light fraction of soil organic matter (LF)) in the ingested soil. The LF represents uncomplexed organic matter that should contribute to C_{min} because LF is not associated with soil minerals and is relatively carbon-rich, compared to other soil organic matter fractions (Janzen et al., 2002).

The initial LF content could predict C_{min} in fresh casts unless endogeic earthworms are selective feeders, meaning that they selectively ingest and/or selectively digest the substrates passing through their gut. A selective ingestion process implies that the endogeic earthworm can isolate and choose to consume fragments of LF, while excluding other particles. Selective ingestion is probably not targeted to particular substrates – earthworms can only ingest particles that fit into their mouth, so choice is restricted by the physical size (referred to as soil fraction size) of the material – but they could choose to ingest a soil fraction size that contains more LF, since this material and other size-density fractions of soil organic matter are not evenly distributed in macro- and micro-aggregates (Janzen et al., 1992; Six et al., 2004; Haynes, 2005). A selective digestion process is aptly illustrated by the concept of "cream skimming", whereby endogeic earthworms assimilate the labile fraction of ingested substrates and egest the remainder, such that their feeding strategy relies upon ingesting a large amount of organic material in a short period of time (Shilenkova and Tiunov, 2015), and soil is ingested without discrimination (Martin et al., 1992; Marhan and Scheu, 2005). If endogeic earthworms engage in selective feeding, this has implications about their ability to process certain soil fraction size(s) to meet their nutritional requirements, and has broader implications for the soil fraction sizes where organic matter accumulate or disappear due to earthworm activities.

The objective was to assess the C_{min} in fresh casts egested by *Aporrectodea caliginosa*, a common endogeic species in temperate agroecosystems, and to relate those biological changes to the initial properties of the soil and soil fractions ingested by the earthworm. In the first experiment, we evaluated the C_{min} of casts, soil impacted by earthworms (surrounding soil) and soil not impacted by earthworms (bulk soil) using three sandy-loam soils (Courval, St. Amable and Chicot soil series) with variable LF concentrations. In the second experiment, our objective was to investigate how the physical size of the ingested soil materials affects the C_{min} after passage through *A. caliginosa*. We hypothesized that gut passage will stimulate C_{min}, although the magnitude of the increase will vary amongst soil fraction sizes. This hypothesis was tested by using six soil treatments obtained from the Courval Soil: whole soil and five soil fraction size classes (2000–1000 µm, 1000–500 µm, 500–250 µm, 250–53 µm and < 53 µm). We used the Courval soil because of its low content of organic carbon, which was expected to induce selective feeding by endogeic earthworms so they could obtain metabolizable carbon at the lowest energetic cost (Martin, 1991; Pilar Ruiz et al., 2006).

2. Materials and methods

2.1. Earthworms and soil preparation

Earthworms (*A. caliginosa*) were collected by hand-sorting in autumn from a grassland system at the University of Vigo Campus (Spain, described by Aira et al., 2010), and transported as passengers in climate-controlled vehicles and commercial airlines to the Macdonald Campus of McGill University where they were kept in large culture boxes (50 l), containing soil moistened to 20% gravimetric moisture content at room temperature (20 °C), for more than two months before starting the experiments. Soils used for earthworm culture and the laboratory experiments were mixed, fine, frigid Typic Endoquents collected from three agricultural fields on the Macdonald Research Farm, Ste-Anne-de-Bellevue, Quebec, Canada (45° 28′ N, 73° 45′ W). All soils were air-dried and sieved (< 2 mm) prior to use in the experiments. Two days before the experiments began, immature individuals (mean fresh weight 0.93 ± 0.15 g) were gently washed and placed on moistened paper to void their gut.

2.2. Experimental design and sampling

This study consisted of two independent laboratory experiments carried out using petri dishes (90 mm × 15 mm) as microcosms for earthworm feeding on soil. Both experiments used completely randomized factorial designs to determine the independent and interactive effects of (1) earthworm gut transit and activity (two levels: with and without earthworms) and (2) soil type or soil size fraction on soil microbial activity, focusing in C_{min} in earthworm casts versus undigested soil as response variable.

In the first experiment, we analyzed the effect of the gut transit (with and without earthworms) and soil type (three sandy-loam soils with variable content of light fraction organic matter: the Courval, St. Amable and Chicot soil series) on the C_{min} of (i) casts egested by earthworms and (ii) surrounding soil, both from microcosms with earthworms, and (iii) bulk soil from microcosms without earthworms as control. Physico-chemical characteristics of the soil fractions are provided in Table 1. Each of the six factorial treatments (with and without earthworms × three soil types) was replicated 20 times, for a total of 120 petri dishes. Each petri dish was filled by 35 g (dry weight) of soil, moistened to 60% of field capacity (about 20% gravimetric moisture content), covered with its corresponding plastic lid and preincubated for a week in a culture chamber at room temperature (20 °C). After the preincubation time, two gut-cleared immature individuals of *A. caliginosa* were added to half of the petri dishes ('with earthworm' treatment). Then, all petri dishes were placed into controlled climate incubators at 17 ± 1 °C in the dark for 2 d. Additional replicated petri dishes (N = 5) with each soil were set for initial soil analyses.

The second experiment used a similar design to investigate the effect of gut transit (with and without earthworms) and, in this case, soil size fractions on C_{min}. Field-collected Courval sandy loam soil was mixed thoroughly after sieving (< 2 mm), and a 5 kg subsample was physically separated by dry sieving (see below) into five soil fraction size classes (2000–1000 µm, 1000–500 µm, 500–250 µm, 250–53 µm