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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 pre-incubated 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

Table 1
Description of three sandy-loam soils under agricultural production used in this study. Mean values (N = 5) are expressed in a dry weight basis.

Soil parameter	Soil series (Crop production)		
	Courval (Corn/ soybean)	St-Amable (Corn)	Chicot (Asparagus)
Sand, g kg^{-1}	629	839	568
pH (H_2O)	6.1	5.4	6.0
Total organic C, g kg^{-1}	13.6	22.2	24.3
Total N, g kg^{-1}	1.28	1.60	1.77
LF, g kg^{-1}	0.70	4.58	1.48
LF-C, g kg^{-1} LF	252	261	283
LF-N, g kg^{-1} LF	39.2	16.8	26.1
DOC, mg kg^{-1}	126	101	92
MBC, mg kg^{-1}	85	176	196
NH_4^+-N, mg kg^{-1}	2.93	1.49	0.70
NO_3^--N, mg kg^{-1}	78.2	20.5	11.0
Basal respiration, µg C–CO_2 h^{-1} g^{-1}	273	540	593

and < 53 µm). A 'control soil' was reconstituted from a subsample of the parent soil that was sieved through the sieving column and then thoroughly remixed. Physico-chemical characteristics of the soil fractions are provided in Table 2. Thus, this second experiment included 12 factorial treatments consisting of two earthworm treatments ('with' and 'without earthworms') × six soil size fraction treatments (control soil plus its five soil size fractions), each replicated 20 times for a total of 240 petri dishes. Procedure for filling and moisturizing the microcosms, earthworm inoculation, temperature and feeding time (2 d) was exactly the same than above and carried out simultaneously.

After 2 d feeding, each petri dish of the 'with earthworms' treatment was sampled for fresh surface casts, hereafter referred to as 'casts'. The 'surrounding soil' that apparently did not pass through the earthworm gut but may have been impacted by the earthworm was collected separately. Fresh casts were carefully retrieved with small forceps under a binocular microscope. Material within these two categories was composited, where one composite sample of casts or one composite sample of surrounding soil was produced by mixing these materials from four randomly-selected petri dishes. This produced five composite samples of casts and five composite samples of surrounding soil per treatment. Composite samples (N = 5) of 'bulk soil' were also made by combining the contents of four randomly-selected petri dishes in the 'without earthworm' treatment.

2.3. Analytical methods

2.3.1. Cast and soil analyses

Initial soils, casts, surrounding soil and bulk soil were analyzed for total organic carbon (TOC) and total nitrogen (TN) using a CN soil analyzer (Flash EA 1112 Series, Thermo-Finnigan Carlo Erba). Moisture content was determined gravimetrically. Mineral N (NH_4^+-N and NO_3^--N) was determined in 0.5 M K_2SO_4 soil extracts (1:5 soil: extractant) and analyzed colorimetrically using the cadmium reduction-diazotization and salicylate methods with a Lachat Quick-Chem AE flow injection autoanalyzer (Lachat Instruments, Milwaukee, WI). The dissolved organic C (DOC) and microbial biomass C (MBC) concentrations in unfumigated and fumigated soil K_2SO_4 extracts was measured by wet combustion with a Shimadzu TOC-V carbon analyzer (Shimadzu Corporation, Kyoto, Japan). The MBC concentration was adjusted using the K_{EC} factor of 0.45 to correct for extraction efficiency (Joergensen, 1996).

Texture of the initial soils was determined in air-dried soil samples of the three soils which were separated into five size classes (2000–1000 µm, 1000–500 µm, 500–250 µm, 250–53 µm, and < 53 µm) by dry sieving in a sieving column (Soil test Inc., Mod. CL-305A, Evanston, Il., US; ca. 450 g soil during 90 s each time). The same procedure was used for preparing the five soil fraction size classes used in the second experiment. Sand content in the three original soils and soil-size fractions was determined after aggregate dispersion with sodium hexametaphosphate (Angers and Mehuys, 1993). The light fraction of the soil organic matter (LF) was isolated and determined after aggregate digestion and isolation by floating in NaI (Janzen et al., 1992). Briefly, a subsample of air-dried soil of known weight was dispersed in a high-density solution of NaI (d = 1.70 g ml^{-1}) and the organic LF quantitatively recovered by flotation on a filter paper using a vacuum pump. LF-C and LF-N concentrations were subsequently determined with the elemental analyser described above.

2.3.2. C mineralization

A subsample of ca. 2 g fresh weight of each initial soil, and of each composite sample of casts, surrounding and bulk soils produced during the 2 d feeding period, was immediately transferred into a 20-mL sterilized serum bottle, sealed with a septa and a metal cap, and placed in a controlled climate chamber (20 °C) for measurement of CO_2–C production after 2 and 4 d.A set of replicated empty bottles and bottles with the equivalent amount of distilled water were included as control. Gas samples from the headspace of the incubation bottles were collected using a gas-tight syringe and injected into pre-evacuated 12 ml exetainer tubes (Labco, High Wycombe, UK) for storage until analysis for CO_2 by a gas chromatograph (GC HP 5890 Series II, Hewlett

Table 2
Characteristics of whole soil and soil fractions with sizes of < 0.53 µm to 1000–2000 µm in diameter in the Courval sandy-loam soil. Mean values (N = 5) are expressed in a dry weight basis. Within a row, values with different letters are statistically different.

Soil parameter		Whole soil	Size fraction (µm)				
	F_{df}, P		2000–1000	1000–500	500–250	250–53	< 53
Sand, g kg^{-1}	$F_{5,12}$ = 993***	632c	585e	595d	689a	664b	1.5f
Total organic C, g kg^{-1}	$F_{5,22}$ = 421***	13.5c	15.2b	15.2b	12.4d	12.1d	25.2a
Total N, g kg^{-1}	$F_{5,22}$ = 62***	1.36bc	1.52b	1.50b	1.34bc	1.24c	2.62a
LF, g kg^{-1}	$F_{5,12}$ = 18.6***	0.72bc	1.27b	2.07a	0.99bc	0.58 cd	0.14d
LF-C, g kg^{-1} LF	$F_{5,12}$ = 0.7 ns	236	246	280	204	248	n.a
LF-N, g kg^{-1} LF	$F_{5,12}$ = 5.1***	11.30c	8.27f	8.55e	9.67d	14.96b	36.13a
DOC, mg kg^{-1}	$F_{5,22}$ = 2.9*	147b	160b	172b	183ab	146b	240a
NH_4^+-N, mg kg^{-1}	$F_{5,22}$ = 7.7***	3.54a	2.81ab	2.97a	2.07bc	1.62c	3.36a
NO_3^--N, mg kg^{-1}	$F_{5,22}$ = 13.4***	64.5b	70.1b	74.7b	72.3b	74.8b	105a
Basal respiration, µg C–CO_2 h^{-1} g^{-1}	$F_{5,22}$ = 344***	304b	281bc	304b	245c	300b	763a

n.a: not available.
ns: not significant.

Packard Co, Avondale, PA, USA) equipped with a thermal conductivity detector at 300 °C. The C_{min} was calculated as the amount of CO_2 evolved relative to the dry mass of soil or cast, and expressed as μg CO_2−C g^{-1} h^{-1}. The first experiment generated 45 bottles for CO_2−C analysis, since there were five replicates of each of the following treatments: casts from three 'earthworm × soil' treatments, surrounding soil from three 'earthworm × soil' treatments and bulk soil from three 'without earthworm × soil' treatments. The second experiment resulted in 90 bottles prepared for CO_2−C analysis because there were five replicates of casts and five of surrounding soil from the six 'earthworm × soil' treatments, plus five replicates of bulk soil from the six 'without earthworm × soil' treatments.

We calculated a normalized carbon mineralization (normalized C_{min}) by subtracting the amount of CO_2 produced in the bulk soil from that released from casts corresponding to the same original soil. We used normalized C_{min} as a proxy of the priming effect of earthworm feeding behaviour and earthworm gut transit and digestive processes on C_{min} to compare across soil treatments.

2.4. Statistical analyses

To ensure that data met the criteria for analysis of variance, normality and homogeneity of variances were tested respectively with Shapiro-Wilk test and Levene's test. Then, the main effects mediated with and without earthworms (in casts, in surrounding soil and in bulk soil) in combination with soil type or soil fractions were evaluated by analysis of variance (ANOVA). Significant differences in the main effects were further analyzed by Fisher's LSD test. To describe the relationships among C_{min} in casts, soil properties and cast properties, we used Pearson's correlation coefficients. All analyses were carried out using SPSS software (IBM SPSS Statistics 20.0), and data presented in the tables and figures is the mean ± standard error (SE).

3. Results and discussion

3.1. C mineralization from casts produced in three sandy-loam soils

There was significantly ($p < 0.05$) more C_{min} in endogeic earthworm casts than surrounding soil and bulk soil, as the casts produced 2 to 3 times more CO_2−C than uningested soils in the 0-2 d incubation period(Fig. 1.a). Similarly, the C_{min} in the 2-4 d incubation period was significantly ($p < 0.05$) greater in casts than surrounding soil and bulk soil (Fig. 1.b). Since the C_{min} was 89% lower in the 2-4 d incubation period than during the first 2 days of incubation, probably due to substrate depletion and drying of the soil and casts (Scheu, 1987; Chaoui et al., 2003), the subsequent analysis focuses on the C_{min} results collected during the 0–2 d incubation period. Casts of endogeic earthworms typically have higher C_{min} than non-ingested soil, which is detected within 2 d after casts are deposited by the earthworm and may persist for up to one year in the case of *A. caliginosa* (Shaw and Pawluk, 1986; Scheu, 1987; Aira et al., 2003, 2005).

The normalized C_{min} for casts, which subtracts the baseline C_{min} flux from the bulk soil, was highest in the St-Amable soil, lower in the Chicot soil and lowest in the Courval soil (Table 3). Casts from the Courval soil had significantly ($p < 0.05$) less TOC and TN than casts produced from the other soils (Table 3). Correlation analyses showed that the normalized C_{min} for casts was associated with initial soil parameters as follows (in Table 1): the LF mass ($r = 0.86$, $p < 0.001$, $n = 15$), the LF-C ($r = 0.87$, $p < 0.001$, $n = 15$), the microbial biomass C concentration ($r = 0.67$, $p = 0.002$, $n = 15$), and the TOC concentration ($r = 0.62$, $p = 0.013$, $n = 15$). These parameters are intercorrelated because they are fractions of the soil organic matter pool. The positive correlations between normalized C_{min}, these soil organic matter fractions and the TOC concentration in casts ($r = 0.73$, $p = 0.002$, $n = 15$; Table 3) are consistent with the observation that C is the primary factor limiting microbial respiration in casts (Scheu, 1987).

The positive correlation between the normalized C_{min} and the LF-C suggests that the LF may be an important source of carbon and energy for endogeic earthworms and microflora in their casts. Decomposing organic matter like LF is part of the diet of *A. caliginosa*, as noted by Zhang et al. (2009), although this species is reported to feed primarily upon humified organic matter (Bouché, 1977). Our results agree with literature postulating a flexible feeding behavior for endogeic earthworms that enables them to adapt to resource availability, such that they can obtain energy and nutrients from both humified and readily assimilable organic compounds in the soil (Martin, 1991; Martin et al., 1992; Tiunov and Scheu, 2004; Marhan et al., 2007; Ngo et al., 2012; Shilenkova and Tiunov, 2015). The relative contribution of humified and partially decomposed organic matter to the diet of *A. caliginosa* is unknown, and whether earthworms choose food sources according to their physiological needs or based on properties of the organic matter warrants further investigation.

In our first experiment, *A. caliginosa* were provided with whole soil that passed through a 2 mm mesh sieve. If earthworms ingested LF in proportion to the LF mass present in whole soil, then earthworm casts would produce the greatest normalized CO_2−C in the soil with the highest g LF-C kg^{-1} LF value. Since this was not the case ($r^2 = 0.04$, $p = 0.49$, $n = 15$), we might suggest that *A. caliginosa* could distinguish and preferentially consume LF from the whole soil. The evidence to support this notion comes from the greater normalized CO_2−C concentration in casts isolated from the St. Amable soil, which possessed a high LF mass and high C:N ratio in LF that could provide more metabolizable substrates to microorganisms in earthworm casts (Fig. 2b). Preferential consumption is also supported by the significant, 23% increase in TOC concentration in the fresh casts relative to the bulk soil from St. Amable series, whereas no significant differences were found in the TOC concentration in fresh casts and bulk soil of the Courval and Chicot series (Table 3). Normally, the C content in casts should be less than the C content of the soil because some C is assimilated as the material passes through the gut. There are three possibilities that could explain the observed increment in TOC in casts of the St. Amable soil relative to the bulk soil. The first explanation is that earthworms are selectively feeding on C-rich organic materials, which increases the TOC concentration in casts. This means that earthworms deliberately select and ingest particles containing LF-C from the St. Amable soil; they digest these substrates efficiently to meet their C and energy requirements, and egest any undigested material in casts. The second explanation is that the LF-C was relatively more degraded or easily metabolized in the St. Amable soil due to the chemical composition of the organic matter derived from the long-term corn monoculture. The third explanation is the earthworm itself adds C to the casts through mucus-C secretion into material passing through the gut. Lavelle et al. (1983) and Trigo et al. (1999) noted that mucus production by earthworms is inversely related to the organic matter content of ingested material, such that earthworms feeding on soil with low organic matter content have higher mucus concentration in casts. This does not match our results since the St. Amable soil with the highest TOC content also had the highest TOC concentration in casts. Also, using the estimated daily loss of C as mucus in cast (0.5% of total animal C) obtained by Scheu (1991) for the temperate endogeic *O. lacteum*, we estimated that mucus-C secretion by *A. caliginosa* would be about 0.1 mg g^{-1} of soil and this does not account for the significant increase in TOC concentration in casts of the St. Amable soil. Since we can rule out mucus-C addition to the casts, we conclude that *A. caliginosa* likely exhibit selective feeding (i.e., selective ingestion and/or digestion processes) of labile organic matter, mostly likely LF. The nuances of how much LF-C is ingested and partitioned during the digestion process (e.g., assimilated into tissue, allocated for mucus production, respired, egested in casts) requires further study, perhaps through natural abundance $\delta^{13}C$ measurements coupled with a source partitioning model.

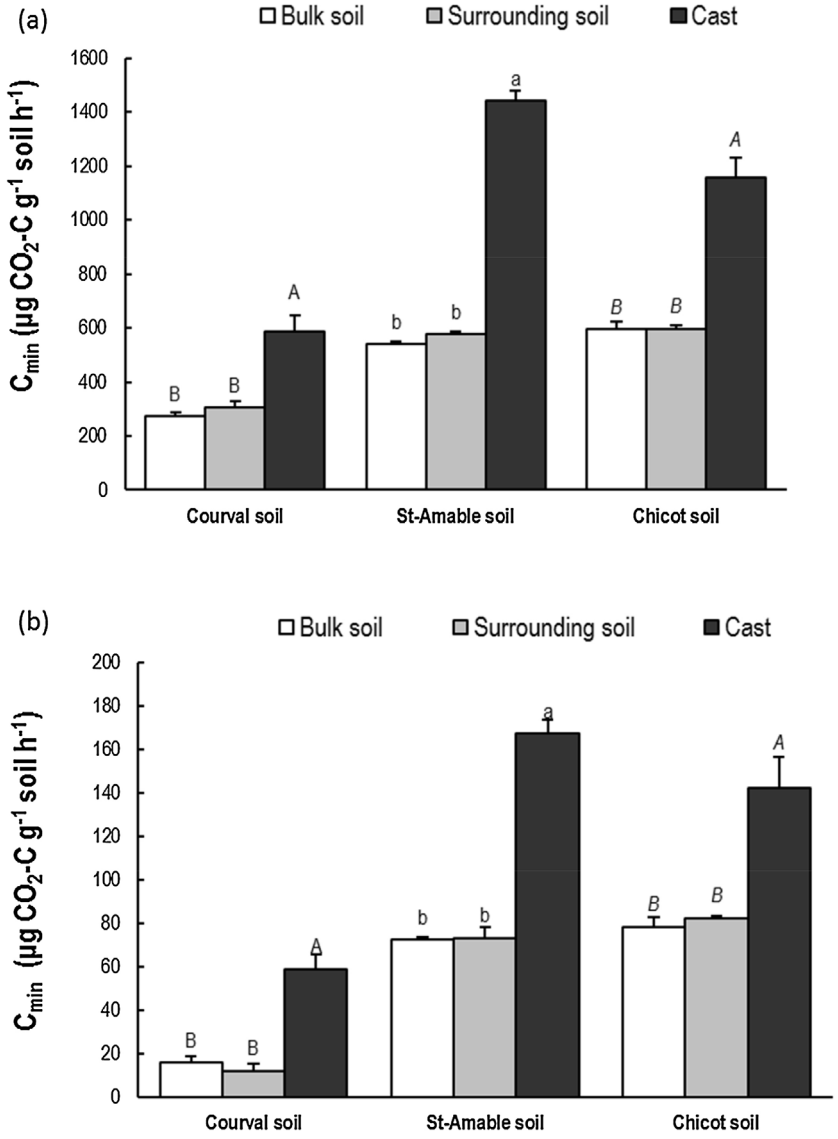

Fig. 1. Carbon mineralization (C_{min}) in earthworm casts, surrounding soil in the vicinity of earthworms and bulk soil unaffected by earthworms in three agricultural soils from 0–2 days (a) and 2–4 days of incubation of the cast and soil materials at 20 °C (b). Mean ± standard error in a dry weight basis. N = 5. Within each soil type, different letters is denoting significant difference between fresh casts and the soil (bulk soil, surrounding soil) at $P < 0.05$.

3.2. The effect of soil fraction size on C mineralization in casts

Casts produced by earthworms feeding on the Courval soil fractions had, on average, 2-fold greater C_{min} than the surrounding and bulk soil. Specifically, the C_{min} in casts compared to bulk soil fractions was 2.2 times more in the 2000–1000 µm fraction, 2.7 times more in the 1000–500 µm fraction, 1.8 times more in the 500–250 µm fraction, 2.3 times more in the 250–53 µm fraction and 1.7 times more in the < 53 µm fraction. This indicates that passage of soil fractions through the gut of *A. caliginosa* always increased the C_{min} in casts relative to the non-ingested soil. The C_{min} in casts was significantly ($p < 0.05$) greater in two soil fractions (1000–500 µm and < 53 µm) than the intermediate fraction of 500–250 µm (Table 4). Similar to the first experiment with three agricultural soils, these effects were related

Table 3
Total organic C, total N, moisture content, and normalized C mineralization (C_{min}) in casts produced by *Aporrectodea caliginosa* and bulk soils unaffected by earthworms from three agricultural soils. Properties of soils are described in Table 1. Normalized C_{min} is calculated by subtracting C_{min} in the bulk soil from C_{min} in the casts and it is a proxy of the priming effect of earthworm ingestion and digestion processes on soil microbial activity. Mean ± standard error, N = 5. For each row, different letters are showing statistical difference between casts across the soil series. Within each soil type, an asterisk (*) is denoting significant difference between fresh casts and the bulk soil ($P < 0.05$, post-hoc LSD test).

Soil series	Courval		St-Amable		Chicot	
	Bulk soil	Cast	Bulk soil	Cast	Bulk soil	Cast
Total organic C (g kg^{-1})	12.8 ± 0.2	12.7 ± 0.2b	18.5 ± 0.5	22.7 ± 0.6a*	22.3 ± 0.6	22.8 ± 0.1a
Total N (g kg^{-1})	1.21 ± 0.02	1.30 ± 0.04c	1.45 ± 0.05	1.68 ± 0.03b*	1.77 ± 0.07	1.82 ± 0.01a
Moisture (g kg^{-1})	0.24 ± 0.003	0.22 ± 0.004b	0.25 ± 0.005	0.24 ± 0.004a	0.24 ± 0.003	0.25 ± 0.002a
Normalized C_{min} (µg CO_2–C g^{-1} h^{-1})	–	312 ± 51c	–	901 ± 29a	–	563 ± 93b

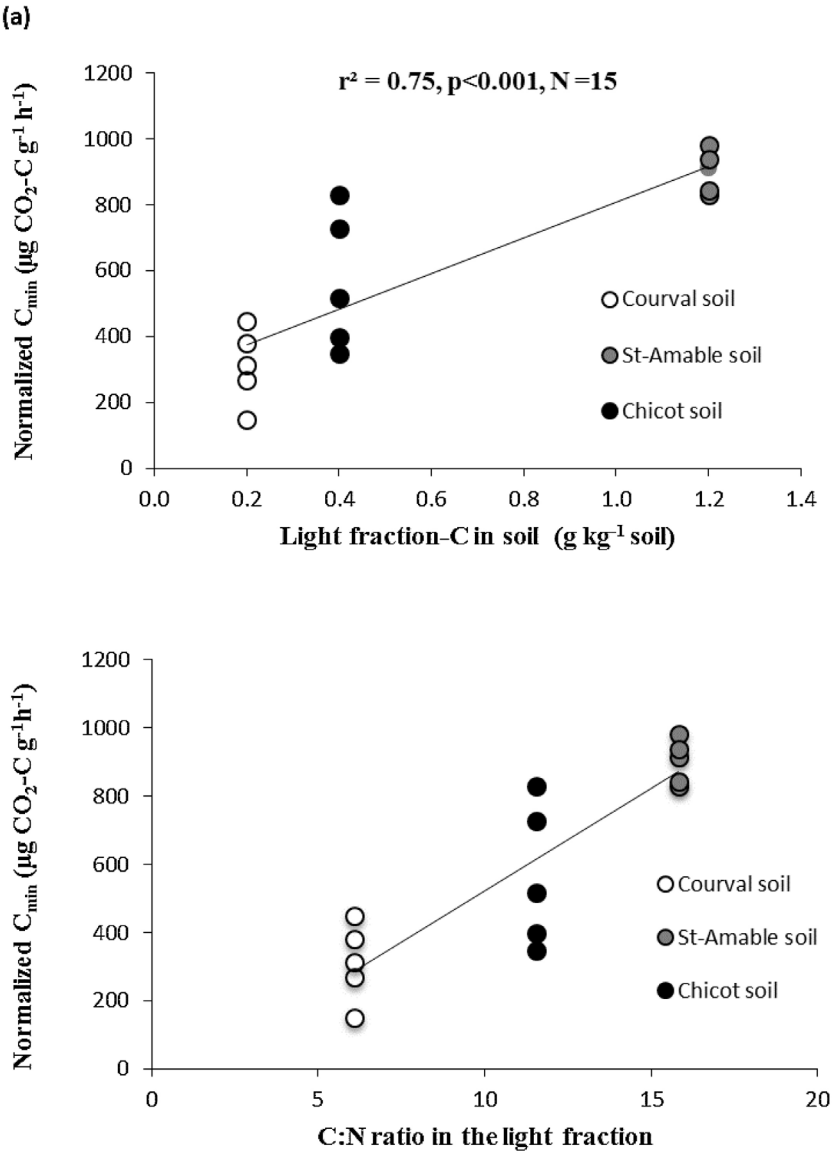

Fig. 2. Priming of carbon mineralization (C_{min}) in earthworm casts (measured as normalized C mineralization; C_{min} in the earthworm casts minus C_{min} in the bulk soil) in three different agricultural sandy-loamy soils as a function of the (a) concentration of carbon in light fractions of soil organic matter (LF-C), and (b) as a function of the quality of such organic matter, measured as the C/N ratio in the light fractions of soil organic matter.

to the initial properties of the soil fraction. For instance, the 1000–500 µm fraction had greater LF content and the < 53 µm contained more TOC and TN than the whole soil and most other soil fractions (Table 2), which appeared to be favorable for C_{min} in casts.

The normalized C_{min} for fresh casts was positively related to the enrichment of LF in soils and soil fractions (r = 0.36, p = 0.079, n = 25), and positively correlated with the LF-C concentration (r = 0.53, p = 0.006, n = 25; Fig. 3). These results agree with those from the first experiment. The normalized C_{min} for casts was lowest for the intermediate soil fraction (500–250 µm) (Table 4), although this fraction had similar LF-C concentration kg^{-1} LF as the other soil fractions (Table 2). If the "cream skimming" analogy can be invoked

Table 4
Total organic C, total N, moisture content, C mineralization, and normalized C mineralization rates in *Aporrectodea caliginosa* casts collected after 2 days of incubation in petri dishes containing five soil fractions (< 0.53 µm to 1000–2000 µm in diameter) or the original whole soil. Normalized C_{min} is calculated by subtracting C_{min} in the bulk soil from C_{min} in the casts and it is a proxy of the priming effect of earthworm ingestion and digestion processes on soil microbial activity. Mean ± standard error, N = 5. For each row, values with different letters are statistically different (P < 0.05, post-hoc LSD test).

	Soil size fraction					
	Whole soil	2000–1000 µm	1000–500 µm	500–250 µm	250–53 µm	< 53 µm
Total organic C ($g\ kg^{-1}$)	13.2 ± 0.24c	14.0 ± 0.16bc	14.8 ± 0.54b	11.9 ± 0.24d	11.8 ± 0.35d	25.2 ± 0.06a
Total N ($g\ kg^{-1}$)	1.27 ± 0.02bc	1.36 ± 0.05b	1.17 ± 0.08cd	1.07 ± 0.08d	1.10 ± 0.06d	2.33 ± 0.10a
Moisture ($g\ kg^{-1}$)	0.27 ± 0.003d	0.29 ± 0.002c	0.31 ± 0.002b	0.28 ± 0.004cd	0.28 ± 0.007d	0.34 ± 0.002a
C_{min} (µg CO_2–C $g^{-1}\ h^{-1}$)	697 ± 67b	622 ± 85bc	807 ± 101b	448 ± 16c	676 ± 53b	1273 ± 58a
Normalized C_{min} (µg CO_2–C $g^{-1}\ h^{-1}$)	394 ± 62ab	341 ± 81ab	504 ± 103a	203 ± 22b	376 ± 62ab	510 ± 60a

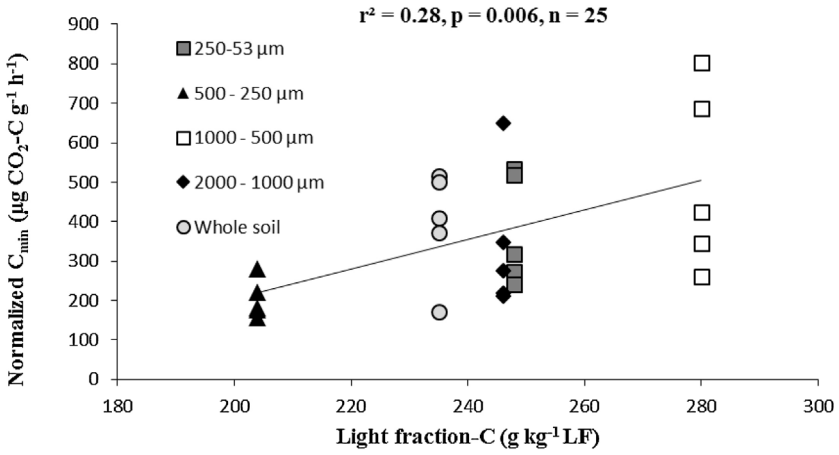

Fig. 3. Priming of carbon mineralization (C_{min}) in earthworm casts (measured as normalized C mineralization; C_{min} in the earthworm casts minus C_{min} in the bulk soil) as a function of the concentration of carbon in the light fractions of soil organic matter (LF-C) in different soil fractions from < 0.53 µm to 1000–2000 µm in diameter obtained from the Courval soil.

here, then some of the LF-C was used for energy/metabolism as the 500–250 µm fraction passed through the earthworm digestive tract, so the casts were depleted in LF-C and thus produced less CO_2—C. As we did not measure the LF-C concentration in casts after the 2 days incubation, we do not have direct evidence to support this argument. However, our findings are partially corroborated by Martin (1991), who found that C content of the 2000–250 µm fraction was reduced by 30% in casts of the endogeic species *M. anomala* relative to the control soil, while no significant difference was observed between the casts and the control soil for organic fractions finer than 250 µm. Previous feeding studies with *A. caliginosa* indicate that the nutritional benefits they derive from well-decomposed organic materials are influenced by the physical size of the organic materials (Curry, 2004; Curry and Schmidt, 2007). Even when *A. caliginosa* was provided with fresh barley straw, which is not a preferred substrate, they grew nearly two times larger when the straw was ground to less than 0.2 mm, than when the size was 0.2–1.0 mm (Boström and Lofs-Holmin, 1986). It is recognized that earthworms, especially endogeic species, rely upon microbially-aided digestion to meet their energy and nutritional demands (Lattaud et al., 1998; Trigo et al., 1999; Brown et al., 2000). As microbial colonization and growth occur more rapidly on finer than coarse materials, this enhanced microbial activity permits endogeic earthworms to derive more metabolizable substrates from finer materials.

If 'finer is better' for endogeic earthworm digestion, a question could be asked about why the 250–53 µm and < 53 µm fractions were not depleted in metabolizable C during gut passage. In fact, casts from the 250–53 µm fraction produced an intermediate level of CO_2—C, whereas casts from the < 53 µm fraction produced the most CO_2—C (Table 4). The residence time and the rearrangement of the ingested material in the earthworm gut can provide an answer to this question. Gut transit time in *A. caliginosa* may vary widely depending on the nature of the material ingested, from 0.77 h estimated by Martin (1982) to 12–24 h found by Piearce (1972) shown in Table S1, and it is possible that the 250–53 µm and < 53 µm fractions pass too quickly through the earthworm gut for the earthworm to deplete the C from these fractions. As well, these fractions may agglomerate into aggregates upon reaction with calcium (released from the earthworm's calciferous glands; Canti and Piearce, 2003), intestinal mucus and polysaccharides of microbial origin during gut passage, and are subsequently deposited as casts containing a considerable amount of potentially metabolizable C, which is then respired as CO_2—C from casts. These possibilities merit further investigation. However, based on the present results, we postulate that the 500–250 µm fraction is the optimal size to support the nutritional requirements of endogeic earthworms, whereas fractions >500 µm are too big and fractions < 250 µm are too small.

4. Conclusions

In summary, our study showed that the magnitude of the short-term positive effect of ingestion and digestion processes of *A. caliginosa* on soil C mineralization is soil-specific, and depends on the initial properties of the ingested soil, such as the content of the LF and the soil-size fraction. This observation requires further testing with soils having a wider range of texture, mineralogy, organic matter content and microbial community composition to improve understanding of endogeic earthworm effects on soil C mineralization at a larger scale. Moreover, by studying one of the most common species in temperate agroecosystems (*A. calginosa*), our work also offered new evidence regarding the feeding behavior of endogeic species. Through our first experiment, we were able to provide a new argument that the LF, a biologically and chemically active pool of soil organic matter, could be part of the diet of *A. caliginosa,* as asserted by Zhang et al. (2009). On one hand, this should lead us to reevaluate the often-repeated claim that endogeic earthworms, including *A. caliginosa*, derive nutrition solely from humified organic substrates or soil humus. By definition, humus is physically and chemically stabilized from biological degradation, thus unlikely to provide a significant nutritional benefit to earthworms. On the other hand, this further supports the idea that endogeic species are flexible in feeding behavior, and can adapt themselves to resource availability by feeding on both humified and readily assimilable organic compounds in the soil. Our results in the second experiment underlined the importance of considering the physical size of soil fractions when evaluating the changes that occur in soils after their passage through the gut of endogeic earthworms. Since endogeic earthworms ingest and produce casts from soil physical fractions of < 53 µm to 2000 µm in diameter, it seems unlikely that they engage in selective ingestion, which implies deliberate choice and active ingestion of particles with specific physico-chemical properties. In contrast, we contended that they might be exerting a selective digestion to obtain energy and carbon from the ingested material. Based on our results, we postulate that the 500–250 µm fraction contains C that is assimilated preferentially by endogeic earthworms and could be the optimal size to support their nutritional requirements. To confirm this assumption, we suggest the use of isotope tracers to reveal if earthworms really assimilate more energy and carbon from these fractions. Our work also highlights the need to consider how the gut transit time affects the digestibility of organic substrates in the earthworm intestinal tract. We suggest that the C_{min} potential of nascent macro-aggregates egested by earthworms (i.e., casts) can be predicted by the initial soil properties and the residence time in the earthworm gut. For instance, higher CO_2 respiration in casts is predicted when endogeic earthworms consume finer, carbon-rich material that passes

through their digestive tract in a few hours compared to coarser, carbon-rich material that remains in the gut for up to one day.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2017.02.022.

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