



## Selective ingestion contributes to the stoichiometric homeostasis in tissues of the endogeic earthworm *Aporrectodea turgida*

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### ABSTRACT

Most detritivores maintain a stoichiometric homeostasis in their body tissue regardless of the chemical composition of the substrates they ingest, but the mechanisms for stoichiometric regulation in soil-inhabiting detritivores like earthworms are poorly understood. The objectives of this study were (1) to examine whether the endogeic earthworm *Aporrectodea turgida* (Eisen) exhibits a strict homeostasis in its tissue C:N ratio, (2) to determine if *A. turgida* controlled its tissue N concentration by changing the quantity of N excreted in epidermal mucus and (3) to consider how the gut transit time, gut load and cast production were related to selective ingestion, which is hypothesized to control the N stoichiometry in *A. turgida* tissues. Two laboratory experiments were designed to address these objectives. In the first experiment, we evaluated the C and N concentrations, and C:N ratio of *A. turgida* body tissue and epidermal mucus after the earthworm fed on soil mixed with <sup>15</sup>N-labeled plant litter (red clover leaves, wheat leaves, wheat stem) having variable N content and C:N ratios, as well as no litter, for 7 days. The second experiment measured the gut transit time, gut load and cast production of *A. turgida* fed soil marked with glass beads, either without litter or mixed with plant litter (soybean leaves with high N, wheat stems with low N). The endogeic earthworm *A. turgida* maintained strict homeostasis in their body tissue, with a C:N ratio of 3.9. The epidermal mucus of *A. turgida* also showed a strict homeostasis (C:N ratio = 4.6) and constant <sup>15</sup>N enrichment, regardless of the N content in plant litter. Therefore, N secretion through epidermal mucus cannot be a mechanism that regulates the N stoichiometry in the body tissue of *A. turgida*. The gut transit time of ingested substrates was the same, as both N-rich (i.e., soil-soybean mixture) and N-poor (i.e., soil-wheat mixture) substrates took  $21 \pm 1$  h to pass from the mouth to the anus of *A. turgida*, however, there was significantly ( $P < 0.05$ ) less material in the gut and less cast production from the N-rich than the N-poor substrate. We conclude that a selective ingestion process controls the intake of organic substrates and likely contributes to the conservation of N stoichiometry in *A. turgida* body tissues.

### 1. Introduction

Stoichiometric homeostasis, the degree to which organisms maintain a constant elemental composition in their body regardless of trophic resources, is a core concept in ecological stoichiometry (Sterner and Elser, 2002). In theory, organisms that maintain strict homeostasis will resist changes in their body composition by releasing elements in excess of their needs and retaining the most limiting elements from trophic resources. This may increase the recycling rate of the non-limiting elements, while depleting the limiting elements from the ecosystem (Vanni, 2002), altering nutrient availability and flows at an ecosystem-level (Sperfeld et al., 2017). For instance, changes in dominant species within a zooplankton community, from a homeostatic consumer with a low N:P ratio (i.e., having an elevated P body content,

thus retaining P in their biomass and releasing N at higher rates) to a homeostatic consumer with a high N:P ratio (i.e., an elevated N body content), caused a shift in phytoplankton growth conditions from P limitation to N limitation in a freshwater ecosystem (Sterner et al., 1992). Primary production is generally limited by N availability in terrestrial ecosystems and particularly in agroecosystems, so populations of detritivorous organisms like earthworms that maintain strict homeostasis in their elemental N content could have important consequences for soil N cycling and crop production.

Earthworms are dominant detritivores in agroecosystems and strictly homeostatic in their C and N contents, as reported by Marichal et al. (2011) who found a constant C:N ratio of 4.1 in *Pontoscolex corethrus*. Similarly, Scheu (1991) and Chen (2013) documented a C:N ratio of 3.8 and 3.7 in lumbricid earthworms of the endogeic functional

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group. The low C:N ratio in endogeic earthworm tissues is interpreted to mean they have a high biological N demand. However, earthworms often increase the amount of plant-available  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  in agroecosystems for the benefit of crops grown in pots and in field plots (Brown et al., 1999; Van Groenigen et al., 2014). From the perspective of stoichiometric homeostasis, it seems contradictory that strictly homeostatic, N-demanding earthworm populations could increase N availability and flows to agricultural crops. Considering their trophic position in the soil foodweb, this observation is logical because endogeic earthworms derive their nutrition from organic N compounds associated with the light fraction of organic matter (Abail et al., 2017) and release plant-available  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  into the environment through cast defecation, urine excretion and mucus secretion, as well as through mortality (Chertov et al., 2017; Whalen et al., 1999). In addition, the ecological engineering activities of earthworms are responsible for soil structure reorganization, organic substrate fragmentation and stimulation of soil microorganisms responsible for N mineralization and nitrification reactions, producing  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  (Bertrand et al., 2015; Blouin et al., 2013). Still, it is not known how endogeic earthworms regulate the stoichiometric homeostasis in C:N ratio of their tissues.

In other aquatic (Frost et al., 2005) and terrestrial invertebrates (Simpson et al., 1995), a number of physiological (e.g., differential digestion, assimilation, excretion) and behavioural (e.g., food selection) mechanisms can be involved in regulating N homeostasis. Physiological mechanisms that may regulate stoichiometric homeostasis occur in the digestion process, after organic substrates are ground in the earthworm gizzard and move into the proctodeum and foregut sections of the intestinal tract (Brown et al., 2000). Here, the secretion of intestinal mucus stimulates digestion by gut microbiota (Lavelle et al., 1995). Intestinal mucus production is negatively correlated with the quality of the ingested substrate (Barois, 1992; Trigo et al., 1999), indicating a differential digestion mechanism. Second, the earthworm may control the N assimilated through the gut wall, into the bloodstream and muscular tissue. Nitrogen assimilation efficiencies of 10–26% were reported for the endogeic *A. tuberculata*, and more N was assimilated when the organic substrate was mixed with glucose (Whalen and Parmelee, 1999). Finally, the earthworm could control the N losses from the body via urine excretion (quantity, urea and  $\text{NH}_4\text{-N}$  concentrations in urine) as well as epidermal mucus secretion, which varies depending on their body condition, handling, defensive action, and other factors (Laverack, 1963). Whalen et al. (2000) suggested that the amount of N excreted in urine-mucus mixture could be higher when earthworms are fed with N-rich materials than when they are provided with N-poor materials, while Needham (1957) noted that 50% of the total daily N loss from earthworm tissues occurs through the secretion of epidermal mucus. Thus, epidermal mucus could be a physiological mechanism to eliminate excess N from the body when earthworms feed on N-rich substrates.

Earthworm behaviour is another factor regulating N homeostasis. Earthworms ingest a variety of trophic resources: decaying plant litter, living and dead roots, animal dung and the microbiota associated with these organic substrates, along with adhering soil minerals. Curry and Schmidt (2007) documented the selective feeding behaviour of earthworms, and their ability to alter ingestion rates according to the quality of organic substrates, where high-quality substrates have a low C:N ratio (i.e., high N content) and a low-quality substrates have a high C:N ratio (i.e., low N content). Earthworms provided with low-quality substrates have greater ingestion rates and more cast production (Flegel and Schrader, 2000; Flegel et al., 1998), suggesting that the earthworm feeding strategy is to increase the amount of material passing through the gut, presumably so they can derive enough N from the low-quality substrate to meet their metabolic requirements. If earthworms rely on selective ingestion to regulate their N homeostasis, the hypothesis is that earthworms will ingest a larger quantity of N-poor substrates than N-rich substrates, and they will defecate more casts when feeding on N-

poor substrates than N-rich substrates. Testing this hypothesis requires knowledge of the mass of substrates ingested and casts produced by earthworms during a period of time. Concurrent measurements of earthworm ingestion and casting rates can only be done in an artificial, soil-free environment (e.g., using the feeding system described by Whalen and Parmelee, 1999). An alternative approach is to evaluate the time for substrates to pass through the earthworm gut (i.e., the gut transit time), which varies from 1 h (Barley, 1959) to 24 h (Pearce, 1972) and is species-specific (Taylor and Taylor, 2014). Gut transit time is suggested to vary due to substrate quality, but no data on this topic was found in the literature, leading to the hypothesis that gut transit time is faster when earthworms consumes N-rich substrates than N-poor substrates.

The objectives of this study were (1) to examine whether the endogeic earthworm *A. turgida* exhibits a strict homeostasis in their tissue C:N ratio, (2) to determine if *A. turgida* controlled their tissue N concentration by changing the quantity of N excreted in epidermal mucus, and (3) to measure the gut transit time, gut load and cast production as indicators of selective ingestion, which is hypothesized to control the N stoichiometry in *A. turgida* tissues. The objectives were evaluated in laboratory experiments where *A. turgida* were supplied with N-rich and N-poor substrates.

## 2. Materials and methods

### 2.1. Soil and earthworm collection

Soil and earthworms used in this study were collected from the top 15 cm of a cornfield at the Macdonald Campus Farm, Sainte Anne de Bellevue, Quebec, Canada (45°28' N, 73°45' W). The soil was a mixed, frigid Typic Endoquent, classified as a Chicot series sandy loam (609 g sand  $\text{kg}^{-1}$ , 246 g silt  $\text{kg}^{-1}$ , 145 g clay  $\text{kg}^{-1}$ ) with 25.7 g organic C  $\text{kg}^{-1}$ , 2.9 g N  $\text{kg}^{-1}$ , and pH ( $\text{H}_2\text{O}$ ) of 5.4. In these fields, *A. turgida* is the dominant endogeic species (Eriksen-Hamel et al., 2009) and it is often numerically dominant in cultivated agroecosystems of Quebec (Reynolds and Reynolds, 1992). Adult *A. turgida* were collected by hand sorting and kept at 20 °C for 3 to 8 wk in culture boxes containing the original field soil, moistened to 20% gravimetric moisture content. Earthworms were used for two separate laboratory experiments (described below). One day before each experiment began, earthworms were removed from the culture boxes, washed and placed on wet filter paper to void their guts at 16 °C. The mean individual fresh weight of earthworms after gut clearance was 628 mg ( $\pm 101$  mg,  $n = 116$ ).

### 2.2. Experimental setup: effect of plant litter on the C and N concentrations and C:N ratio in *A. turgida* tissue and mucus

The first laboratory experiment evaluated the C and N concentrations, and C:N ratio of *A. turgida* body tissue and epidermal mucus after feeding on  $^{15}\text{N}$ -labeled plant litter with variable N content and C:N ratio. To obtain  $^{15}\text{N}$ -labeled litter, wheat and red clover were grown from seeds in a greenhouse, and fertilized using a  $^{15}\text{N}$ -enriched nutrient solution made up of 10%  $^{15}\text{N-KNO}_3$  (98 atom %  $^{15}\text{N}$ ) and 90%  $\text{KNO}_3$  (0.367 atom %  $^{15}\text{N}$ ). After 7wk, plants were harvested, rinsed with distilled water, oven dried (40 °C for 3 d), and ground homogeneously with a Wiley mill (< 1 mm mesh). Subsamples were analyzed for C and N content, and  $^{15}\text{N}$  enrichment. The N content and C:N ratio varied depending on the litter type: red clover leaves had 47.3% C, 5.7% N, C:N = 8 and atom %  $^{15}\text{N}$  = 5.2, while wheat leaves (46.6% C, 3.2% N, C:N = 15, atom %  $^{15}\text{N}$  = 4.7) and wheat stems (44.0% C, 1.5% N, C:N = 29, atom %  $^{15}\text{N}$  = 5.0) contained less N and a higher C:N ratio. The acid unhydrolyzable fraction (Van Soest et al., 1991), a proxy for lignin content, was similar among litter types: 50 g  $\text{kg}^{-1}$  in red clover leaves, 54 g  $\text{kg}^{-1}$  in wheat leaves and 58 g  $\text{kg}^{-1}$  in wheat stems.

The experiment was a completely randomized design with four treatments: red clover leaves, wheat leaves, wheat stems, and a control

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