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Estimating energy costs of earthworm burrowing using calorimetry

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ABSTRACT

Earthworm burrowing is essential for soil functioning in temperate climates. It is known that soil compaction hampers earthworm burrowing, but there is a lack of knowledge on how it affects the energy costs of earthworms. In the present study, we used respirometry and isothermal calorimetry to quantify earthworm respiration rates and heat dissipation in two endogeic species, *Aporrectodea caliginosa* and *Aporrectodea tuberculata*, in compacted and non-compacted soils. We put the measured respiration rates and heat dissipation in relation to the burrow volume and cast volume produced by the earthworms. We found that at higher compaction levels, respiration rates and dissipated heat increased for both studied species. The energy costs associated with burrowing were a significant fraction of the total energy costs. Our results indicate that energy costs per burrow volume increase due to compaction, and that the specific energy costs for burrowing (i.e., per gram earthworm) were lower for *A. tuberculata* than for *A. caliginosa*. Further studies are needed to confirm our results. We discuss the potential and current limitations of isothermal calorimetry as a method for direct quantification of energy costs of earthworms. There is a need for further studies that quantify how energy costs of burrowing are affected by various soil conditions, to better predict the implications of land use and soil management on soil processes and functions mediated by earthworm burrowing.

1. Introduction

The positive effects of earthworm burrowing on soil processes and soil functions and the contributions of earthworms and their burrowing activity to soil ecosystem services are well known [1–3]. Macropores created by earthworms improve water infiltration, drainage, and gas exchange between the soil and the atmosphere, and the burrows can provide paths of least resistance for roots to reach deeper soil layers [4–7]. Earthworms produce casts, both on the soil surface and within the soil, and mix soil, which has a positive impact on soil aggregation, nutrient availability [8–11], and the physical protection of soil organic carbon [12,13]. The mass of soil transported and mixed by earthworms can amount to several tons of soil per hectare and year [14], which makes earthworms key players in biotic processes in temperate soils.

Earthworms are highly sensitive to soil environmental conditions, including chemical conditions (e.g. soil pH, soil oxygen levels, concentrations of contaminants) and soil physical conditions such as soil moisture, temperature and soil compaction [15–18]. The soil environmental conditions affect earthworm community structure (i.e., species composition and abundance) and earthworm behaviour, and hence burrowing rates (i.e., how fast and how far earthworms move in soil) and total amounts of bioturbation (i.e., how much soil is transported and mixed by earthworms). In the present study, we focused on the effect of soil compaction, which is one of the main threats to soil functioning in modern agriculture [19]. Compaction affects earthworm burrowing directly by enhanced soil mechanical resistance [20–23], and indirectly by modifying soil aeration, and hydraulic and thermal soil properties. In the long term, compaction may also decrease soil carbon inputs from root growth [24,25] and thereby the energy available to earthworms.

An increase in soil mechanical resistance reduces burrowing rates [23] and is predicted to increase the energy requirements of earthworms for burrowing [26]. However, quantitative data on energy costs for burrowing are lacking. Moreover, an increase in compaction may change the mode by which earthworms burrow through soil: burrowing

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by ingestion increases and burrowing by cavity expansion (earthworms pushing the soil aside) decreases when soil mechanical resistance increases [20,23]. The different burrowing modes may be associated with different energy costs, as suggested by Beylich et al. [27], but it remains unclear why earthworms change towards more ingestion when soil becomes harder. Combined data on burrowing rates and energy requirements of earthworms at different compaction levels are needed to shed light on the relationship between burrowing mode and energy costs.

A widely used indirect quantification of an organism's metabolic rate, i.e. the amount of energy used per unit of time, is the measurement of respiration rates that are obtained either from oxygen consumption or carbon dioxide production [4]. Several studies have used respiration measurements to quantify earthworm metabolic rates and how they are affected by environmental conditions [28–30] such as concentrations of heavy metals, contaminants, and nanoparticles [31–33], and temperature [34,35]. Data on respiration rates can be converted to energy consumption by assuming respiration quotients and oxyenergetic coefficients (e.g. Meehan, 2006 [36]). Respiration measurements are therefore sometimes referred to as indirect calorimetry.

A direct quantification of the energy used by an organism can be obtained from calorimetry, also referred to as direct calorimetry. In isothermal calorimetry, the dissipated heat is measured. This technique is used in soil microbiology to quantify energy costs of biological processes with high precision and high temporal resolution [37–39]. While recent years have seen increased use of calorimetry in soil microbiology, direct quantification of energy costs of earthworms has so far received little attention. One exception is the study by Lamprecht (2013) [40], who used a Calvet-type calorimeter to measure heat dissipation from earthworms after being exposed to pentachlorophenol (PCP).

Measurements of respiration rates (indirect calorimetry) and heat dissipation (isothermal calorimetry) can provide information needed to better understand how earthworm metabolic rates and energy requirements are affected by soil properties and soil conditions. Yonemura et al. [41] identified a need for quantitative information on the respiration dynamics of earthworms, i.e., quantifying separately the respiration associated with earthworm maintenance (also referred to as basal respiration rate) and with burrowing (i.e. when earthworms move through soil). Currently, data on energy costs for burrowing are lacking and, hence, it is not known how these costs are affected by soil environmental conditions, and how they differ between earthworm species.

The overall aim of the present study was to quantify the energetics of earthworm burrowing as a function of soil mechanical resistance for two endogeic earthworm species (*Aporrectodea caliginosa* and *Aporrectodea tuberculata*). To achieve this, we performed experiments with a respirometer and an isothermal calorimeter. A specific aim was to test whether isothermal calorimetry could be used to measure the energy requirements of earthworms during burrowing in soil. In addition to obtaining a direct measure of energy costs with isothermal calorimetry, the measurement frequency is high in a calorimeter. Hence we also expected to gain insights into temporal patterns of earthworm activity and diurnal cycles. The experiments in the respirometer served two main purposes: i) to obtain data that can be directly compared with available literature data, and ii) to compare energy costs from calorimetry to data from respirometer measurements using common values for the respiratory quotient and the oxyenergetic coefficient. We hypothesized that the energy costs of burrowing increase in compacted soil, and that the energy costs differ between the two earthworm species.

2. Materials and methods

2.1. Study design

This study considered two earthworm species, *A. caliginosa* and *A. tuberculata*. During a period of 48 h, respiration rates or heat dissipation rates of the earthworms were quantified at two levels of soil compaction (bulk density of 1.0 and 1.4 g cm^{-3}). Respiration and heat dissipation rates were then related to burrow and cast volumes produced by the earthworms. Respiration rates were obtained by measuring the evolution of CO_2 in a RESPICON 96 respirometer [42]. Energy costs were quantified by measuring heat dissipation in an isothermal calorimeter (TAM Air). The experimental setups and procedure were similar for respiration and heat dissipation measurements (Fig. 1). The two methodological approaches will be referred to as "respirometry" and "calorimetry" in the following text. After the measurements in the respirometer and calorimeter, the soil jars were scanned using X-ray computed tomography to quantify burrow and cast volumes (Fig. 1).

In addition to the measurements with earthworms burrowing in the soil, we also made respiration and heat dissipation measurements for soil only (without earthworms) to quantify the contribution from soil microbes and for earthworms only (without soil) to quantify earthworm basal metabolic rates. The earthworms were placed on moist filter paper for the earthworm-only measurement. To obtain the respiration rates and energy costs associated exclusively with earthworm burrowing (referred to here as respiration rates and energy costs of burrowing), we subtracted the respiration or heat associated with the soil (samples with soil only) and the earthworm maintenance costs (earthworm only, basal metabolic rates) from the total respiration or dissipated heat from samples with soil and earthworm. Further details of the calculations are given in the Supplementary Material.

2.2. Soil and soil moisture conditions

The soil was taken from the top 20 cm of an arable field in Uppsala, Sweden (59.8°N, 17.6°E). The texture class is silty clay loam, with 39 % clay, 52 % silt and 8 % sand. The soil organic carbon (SOC) content was 2.3 %, and the pH was 5.9 (measured in a 1:2 ratio of soil: deionized H_2O). Visible plant and root residues were removed before the soil was sieved using a 2 mm mesh. The soil was then oven-dried for 24 h at 105 °C. Deionized water was added to achieve a matric potential of −100 hPa. This matric potential was selected to ensure there was no soil moisture stress for the earthworms. The amount of water required was calculated from water retention measurements carried out on soil cores placed on a tension plate using the same soil and the two dry bulk densities, ρ, considered ($\rho = 1.0$ and 1.4 g cm^{-3}).

2.3. Earthworms

Two earthworm species were used: *Aporrectodea caliginosa* (Savigny, 1826) and *Aporrectodea tuberculata* (Eisen, 1874). *A. caliginosa* is considered endogeic with traits of 80 % endogeic, 4 % anecic and 16 % epigeic [43], and *A. tuberculata* is a larger endogeic species that is commonly found in northern Europe [44–46]. Adult earthworms were collected from an arable field near Uppsala, Sweden. The earthworms were identified using the keys from Andersen (1997) [47] and Hale (2007) [48]. All earthworms were kept in a growth chamber at 20 °C before the experiments. Before the start of the experiments, earthworms were kept in Petri dishes with moist filter paper for 24 h to empty their guts, and then their body mass was recorded. The initial average biomass of earthworms used in the calorimeter and respirometer experiments were 0.48 g (standard deviation, SD = 0.06) and 0.49 g (SD = 0.08) for *A. caliginosa* and 1.06 g (SD = 0.16) and 1.06 g (SD = 0.24) for *A. tuberculata*.

2.4. Isothermal calorimetry

The isothermal calorimeter used for the heat dissipation measurements was a TAM Air (TA instruments). Measurements were carried out at a temperature of 20 °C 50 g of moist soil was placed in a glass jar (125 ml). Soil volumes within the jars were 59 and 36 ml for non-compacted and compacted soil, respectively, leaving an air volume of 66 ml and 89 ml in the jars. Pre-tests prior to the experiments ensured that there were

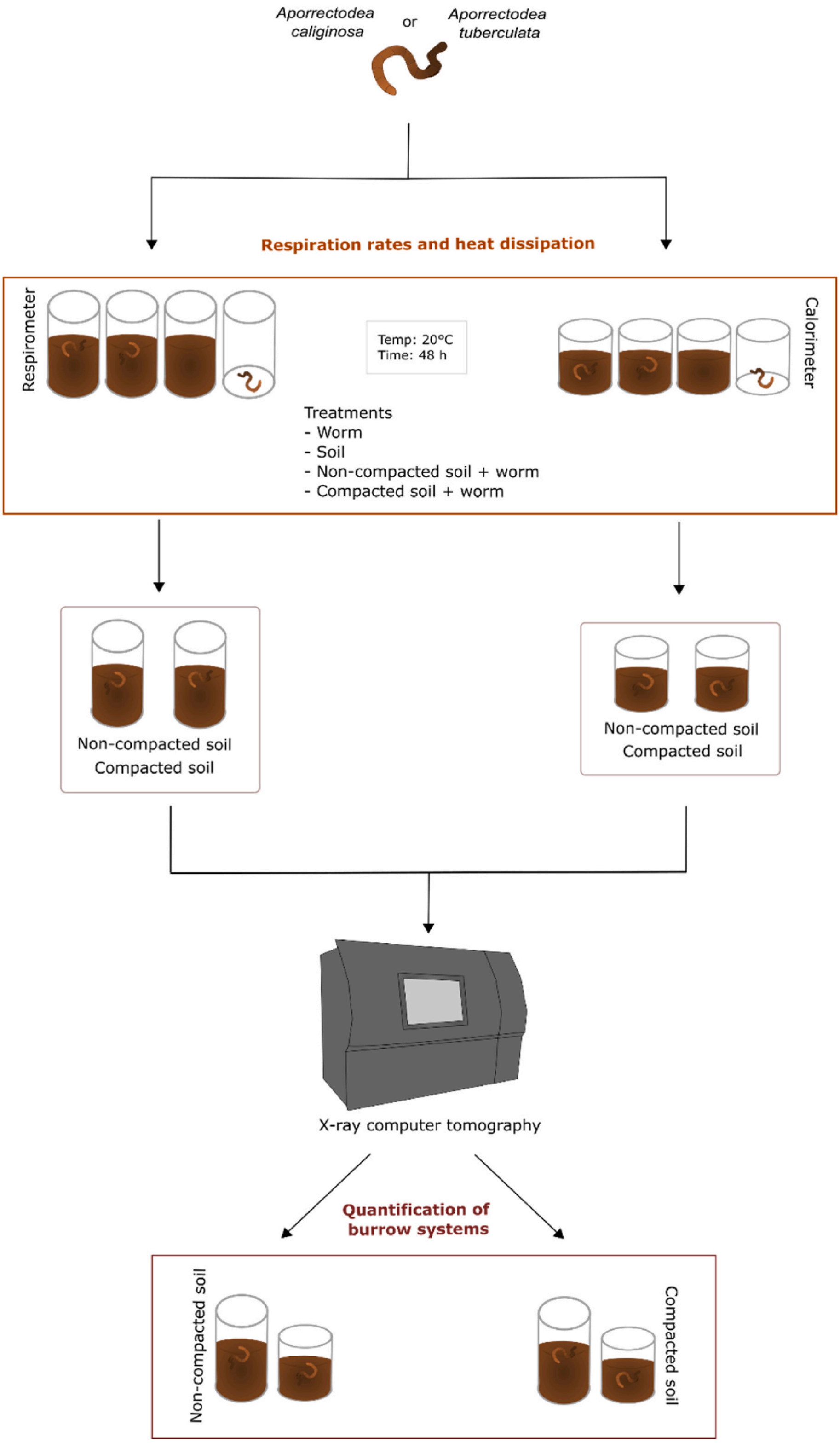

Fig. 1. Schematical overview of the experimental design and illustration of workflow. Respiration measurements: $n = 6$; Heat dissipation measurements: $n = 4$.

sufficient oxygen levels for the entire duration of the experiments (48 h). All soil jars were pre-incubated for a week at a temperature of 20 °C in a growth chamber (Model SED-41C8, Percival Scientific Inc.), the same temperature that was used in the isothermal calorimeter. Earthworms were added to the samples just before placing the jars in the calorimeter.

The earthworms were exposed to either non-compacted soil ($\rho = 1$ g cm^{-3}) or compacted soil ($\rho = 1.4$ g cm^{-3}) for 48 h. For the calorimetry experiment, we had four replicates per compaction level and earthworm species, resulting in a total of 16 experimental units (two compaction levels × two earthworm species × four replicates). In addition, we used four samples with soil only, and three samples with earthworms only for each species.

2.5. Respirometer measurements

For the respiration measurements, we used a RESPICOND 96 as described by Nordgren (1988) [42]. 80 g of moist soil was placed in a plastic jar (250 ml) and pre-incubated under the same conditions as described above for the isothermal calorimetry. Soil volumes within the jars were 105 and 75 ml for non-compacted and compacted soil, respectively, leaving an air volume of 145 ml and 175 ml. As for the calorimetry, pre-tests ensured that there was sufficient oxygen for the entire duration of the experiments. The respirometer experiments were carried out at 20 °C. Compaction levels, earthworm species, and samples with soil only and earthworm only were carried out as described above for the isothermal calorimeter. However, in the respirometer, we used six replicates per compaction level and earthworm species, four replicates with soil only, and four replicates with earthworm only for each species.

2.6. X-ray computed tomography, image processing and analysis

All soil jars were scanned using a GE Phoenix X-ray scanner (v|tome|x 240). 1200 radiographs were collected for each scan at a voltage of 140 kV, a current of 560 μA, and an exposure time of 166 ms. A 3D image was constructed from the radiographs using the GE reconstruction software datos|x 2.1. RTM. The voxel edge length of the 3D images was 40 μm in all directions.

The image processing was carried out using ImageJ with plugins distributed in FIJI [49] and SoilJ [50]. First, a 3D median filter was applied (radius: one voxel) to reduce noise in the images. To segment the solids from the air, the Otsu method was applied [51]. The exact position of the soil surface could not be determined for all samples due to collapsed burrows. Therefore, the soil surface was defined as the vertical slice where the soil covered more than 90 % of the area. This corresponded to a height between 7 and 11 mm for the different treatments.

Earthworms were still present in some of the jars at the time of X-ray scanning. In these cases, segmentation was done by removing voxels with grey values outside the interval 23000 to 35000. The resulting binary image was then eroded (twice) and dilated (twice) to remove partial volume effects (boundaries between air and solids). Objects smaller than 5000 voxels were removed using the “objects counter” in FIJI. Finally, the “3D fill holes” function in FIJI was used to fill some parts of the worm image. As this segmentation method did not remove the entire worm, manual removal of the earthworms was done slice-by-slice.

Earthworm casts had similar grey values as the soil and could not be segmented using a single threshold. Casts within the bulk soil were therefore quantified using a semi-automatic approach, where we applied the “morphological segmentation” tool implemented in MorphoLibJ [52] to 3D gradient images of the samples, followed by manual selection of the resulting watersheds that corresponded to casts. Details are given in the Supplementary Material. Binary images of casts and earthworms were subtracted from the images of solids to get an image of macropores (Supplementary Material Fig. S1). Pores created by earthworms were assumed to have diameters larger than 2.5 mm. All pores with smaller diameters were removed based on local thickness to achieve an image of the burrow network. We used the PoreSpaceAnalyzer from SoilJ [50] to quantify the burrow volume.

2.7. Quantification of cast volumes and estimation of ingestion factor

Where present, the above-surface cast was collected from each sample (surface casts were not produced in all samples) and oven-dried at 105 °C for 24 h. Below-surface casts were identified and their volume was quantified as described in Section 2.6. To estimate cast mass, we used the density of the casts assuming a linear relationship between grey values in the images and the bulk density of the casts and soil (e.g. Pulido-Moncada et al. [53]).

Based on the data of cast volume and burrow volume, we estimated an ingestion factor as described in Arrázola-Vásquez et al. (2022) [23]. The ingestion factor is defined as the fraction of ingested soil volume compared to the total burrow volume. An ingestion factor of zero means that all burrows were created by pushing the soil aside, while an ingestion factor of one indicates that all burrows were created by ingestion.

2.8. Statistical analysis

A generalized linear model (GLM) with soil compaction level and earthworm species as factors was used. We did not consider interaction effects. The data set was too small to check for normality. We compared the means of each parameter analysed to test the effects of either soil compaction or earthworm species. The statistical analysis and figure creation were done in R using the emmeans package version 1.8.4.1 and ggplot2 package version 3.3.3. In this study, p-values below 0.05 were considered significant and p-values in the range of 0.05–0.1 were considered a trend.

3. Results

3.1. Respiration rates

Basal respiration and respiration rates of earthworms are given in Tables 1 and 2. The mean basal respiration rates of *A. caliginosa* and *A. tuberculata* were similar, with values of 0.42 and 0.46 mg CO_2 d^{-1} (Table 1), respectively. For *A. tuberculata*, the basal respiration rate is uncertain and based on one replicate only (in the other replicates earthworms had died).

Respiration rates related to burrowing indicate that *A. tuberculata* respired about twice more than *A. caliginosa* ($p = 0.04$) (Table 2). For both species, respiration rates were almost 50 % higher in compacted than in non-compacted soil, although the difference was not statistically significant ($p = 0.11$). When comparing total respiration rates (Table 1) with respiration rates for burrowing (Table 2), we found that for *A. tuberculata*, 50–70 % of the respiration was associated with burrowing, while for *A. caliginosa* this number was 25–50 %. For both species, the ratio of respiration for burrowing to total respiration was higher in compacted soil. The specific respiration rates for burrowing (i.e., normalized by body mass; Table 2) were higher in compacted than in non-compacted soil ($p = 0.04$). Specific respiration rates for burrowing were not significantly different between earthworm species ($p = 0.65$).

3.2. Heat dissipation

Basal energy costs for *A. tuberculata* were twice as high as for *A. caliginosa* (Table 1), while the specific basal energy costs (i.e., normalized by body mass) were similar for *A. caliginosa* (28 J g^{-1} fw d^{-1}) and *A. tuberculata* (26 J g^{-1} fw d^{-1}). The energy costs for burrowing (Table 2) showed a similar pattern as the respiration rates for burrowing. The energy costs for burrowing did not differ between species ($p = 0.77$). Higher values were measured in compacted soils (around 30 J d^{-1}) compared to non-compacted soils (around 20 J d^{-1}) for both species, but there was no statistically significant difference between compaction levels ($p = 0.33$). Energy costs of burrowing accounted for about 40–50 % of the total energy costs for *A. tuberculata* and about 60–70 % for *A. caliginosa* (Table 2). For both species, specific energy costs for burrowing were higher in compacted compared to non-compacted soil (Table 2), but there was no statistically significant difference between compaction levels ($p = 0.41$). Specific energy costs for burrowing were higher for *A. caliginosa* than for *A. tuberculata* ($p = 0.07$).

Fig. 2 compares data from the calorimeter measurements and the respirometer measurements. We found a linear relationship between heat dissipation and respiration rates. The slope of the regression line

Table 1
Respiration rates and energy costs of earthworm only (basal respiration rate and basal energy costs) and total respiration rates and energy costs of earthworms in the soil, for *A. caliginosa* and *A. tuberculata*. Mean values and standard error (SE). (Respiration: $n = 6$; Energy costs: $n = 4$).

Soil compaction level	Species	Basal respiration (mg CO_2 d^{-1}) R_{worm}	Specific basal respiration (mg CO_2 g^{-1} fw d^{-1}) RB_{worm}	Total respiration, earthworm in soil (mg CO_2 d^{-1}) $R_{Worm\ in\ soil}$	Basal energy costs (J d^{-1}) Q_{worm}	Specific basal energy costs (J g^{-1} fw d^{-1}) QB_{worm}	Total energy costs, earthworms in soil (J g^{-1} fw d^{-1}) $Q_{Worm\ in\ soil}$
	A. caliginosa	0.42 (0.05)	0.74 (0.08)		12.1 (4.0)	28.0 (2.7)	
	A. tuberculata	0.46	0.54		24.8 (15.3)	26.4 (13.3)	
Compacted	*A. caliginosa*			0.85 (0.06)			42.4 (7.3)
	A. tuberculata			1.46 (0.19)			53.0 (11.1)
Non-compacted	*A. caliginosa*			0.55 (0.18)			33.6 (13.5)
	A. tuberculata			1.01 (0.38)			43.2 (3.5)

Table 2
Respiration rate and energy costs for burrowing, for *A. caliginosa* and *A. tuberculata*. Mean values and standard error (SE). (Respiration: $n = 6$; Energy costs: $n = 4$).

Soil compaction level	Species	Respiration rate (mg CO_2 d^{-1}) – burrowing $R_{Burrowing}$	Specific respiration rate (mg CO_2 g^{-1} fw d^{-1}) – burrowing $RB_{Burrowing}$	Energy costs (J d^{-1}) – burrowing $R_{Burrowing}$	Specific energy costs (J g^{-1} fw d^{-1}) – burrowing $QB_{Burrowing}$
Compacted	*A. caliginosa*	0.43 (0.08)	1.15 (0.32)	30.3 (7.3)	57.1 (14.6)
	A. tuberculata	1.00 (0.23)	0.87 (0.24)	28.1 (11.1)	21.2 (11.0)
Non-compacted	*A. caliginosa*	0.13 (0.21)	0.36 (0.43)	21.5 (13.5)	36.8 (23.6)
	A. tuberculata	0.55 (0.47)	0.38 (0.41)	18.3 (3.5)	16.6 (2.5)

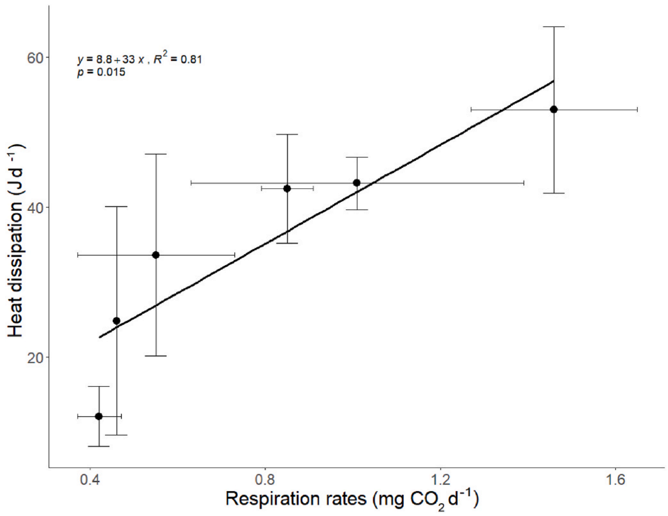

Fig. 2. Relationship between heat dissipation measured in the calorimeter and respiration rates measured in the respirometer, for *A. caliginosa* and *A. tuberculata*. The data points are the mean values of the total respiration/energy costs of earthworms in soil and the basal respiration/energy costs (Table 1). Mean values and standard error bars.

was 33 J per mg CO_2, indicating that 1 mg respired CO_2 d^{-1} corresponded to a dissipated heat of 33 J d^{-1}.

3.2.1. Temporal dynamics of heat flow from earthworms

The temporal dynamics of heat flow for both earthworm species and soil compaction levels are shown in Fig. 3. The measurements show higher and lower activity periods in all individuals, but it was not possible to identify diurnal cycles, general species-specific patterns or characteristic differences between compaction levels. Nonetheless, the data reveal differences between individuals, both in the level of heat dissipation and in the dynamics of heat dissipation. In general, *A. tuberculata* showed more fluctuations in heat dissipation in comparison to *A. caliginosa*. For *A. tuberculata*, heat dissipation peaks were less abrupt and the peak values were slightly higher in the compacted treatment than in the non-compacted treatment. For *A. caliginosa*, activity peaks were more frequent in the compacted than in the non-compacted treatment.

3.3. Burrow and cast volumes

Across both methods (calorimetry and respirometry), *A. caliginosa* created a smaller mean burrow volume than *A. tuberculata*, in both compaction levels ($p < 0.01$) (Table 3). For both species, the burrow volumes in compacted soil were significantly smaller ($p = 0.02$) than in non-compacted soil.

Surface casts were primarily found in compacted soil and generally absent in non-compacted soil. In the non-compacted soil, we only found some surface casts in four replicates of *A. tuberculata*, while in all other non-compacted samples, no surface cast was found. Total cast volume (including both below and above-ground casts) was about 40 % (compacted soil) to 75 % (non-compacted soil) larger ($p = 0.007$) for *A. tuberculata* than for *A. caliginosa* (Table 3). Soil compaction level had no significant effect on cast production ($p = 0.56$).

On average, *A. caliginosa* ingested more soil in the compacted treatment (average ingestion factor, $f_{\text{ingestion}}$, of 0.73) compared to the non-compacted treatment ($f_{\text{ingestion}} = 0.42$) (Table 3). In contrast, *A. tuberculata* showed no change in the ingestion factor between non-compacted soil ($f_{\text{ingestion}} = 0.58$) and compacted soil ($f_{\text{ingestion}} = 0.6$). However, differences were not statistically significant for either species ($p = 0.92$) or compaction levels ($p = 0.22$).

3.4. Energy costs per burrow volume and cast volume

Energy costs per burrow volume (Fig. 4a) were higher for *A. caliginosa* (compacted soil: 29.7 J cm^{-3}; non-compacted soil: 10.3 J cm^{-3}) than for *A. tuberculata* (compacted soil: 12.8 J cm^{-3}; non-compacted soil: 6.1 J cm^{-3}). While the results suggest differences in the energy costs per burrow volume between the species, the differences were not statistically significant ($p = 0.15$). For both species, energy costs per burrow volume were higher in compacted soil ($p = 0.065$).

Energy costs per cast volume (Fig. 4b) followed a similar trend as the energy costs per burrow volume. *A. caliginosa* had higher energy costs per cast volume, with mean values of 40.1 J cm^{-3} (compacted soil) and 16.7 J cm^{-3} (non-compacted soil), in comparison to *A. tuberculata* with mean values of 20.8 J cm^{-3} and 10.1 J cm^{-3} for compacted and non-compacted soil, respectively. Differences between species were not statistically significant ($p = 0.31$). The energy costs per cast volume were about two times higher in compacted soil than in non-compacted soil for both species ($p = 0.13$).

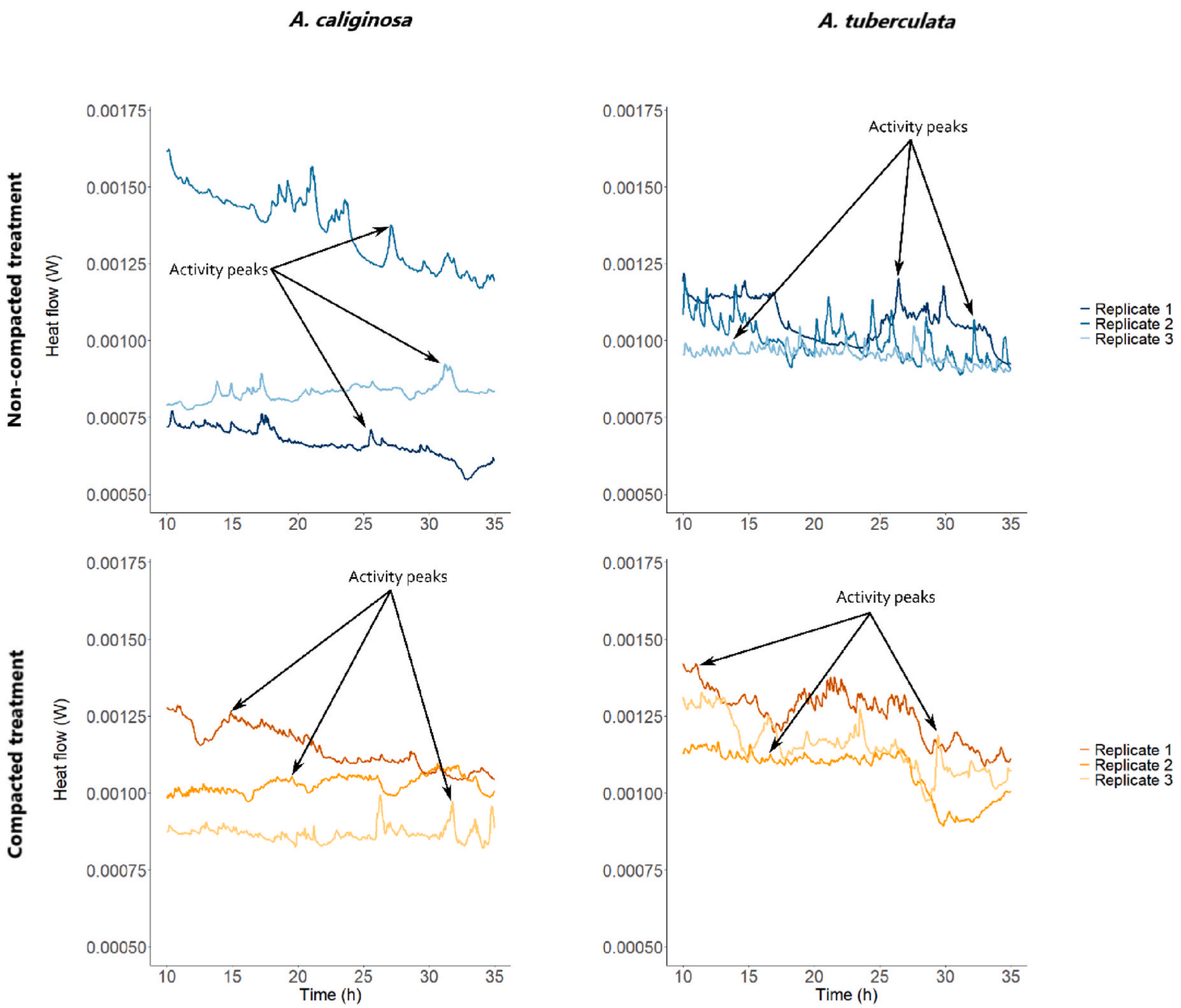

Fig. 3. Temporal dynamics of heat dissipation (W) for *A. caliginosa* and *A. tuberculata* in compacted and non-compacted soil, during one day (from time $t = 10$ to $t = 35$ h).

Table 3
Mean burrow volume, cast volume and ingestion factor for *A. caliginosa* and *A. tuberculata*. Cast volumes include both surface casts and below-ground casts. Mean values and standard error (SE). ($n = 10$).

Soil compaction level	Earthworm species	Burrow volume (cm^3 d^{-1})	Specific burrow volume (cm^3 g^{-1} fw d^{-1})	Cast volume (cm^3 d^{-1})	Specific cast volume (cm^3 g^{-1} fw d^{-1})	Ingestion factor
Compacted	*A. caliginosa*	1.38 (0.13)	2.92 (0.27)	0.81 (0.13)	1.77 (0.29)	0.73 (0.20)
	A. tuberculata	2.11 (0.20)	1.94 (0.19)	1.12 (0.15)	1.05 (0.15)	0.60 (0.11)
Non-compacted	*A. caliginosa*	1.85 (0.12)	3.79 (0.20)	0.77 (0.16)	1.53 (0.29)	0.42 (0.08)
	A. tuberculata	2.73 (0.35)	2.50 (0.26)	1.34 (0.17)	1.34 (0.23)	0.58 (0.12)

4. Discussion

We quantified respiration rates and measured heat dissipation of two earthworm species burrowing in the soil at two levels of compaction. Respiration rates obtained in our study were similar to values reported in the literature, while there were no values for heat dissipation available for comparison. Total respiration rates of *A. caliginosa* in our study (1.1–1.7 mg CO_2 g^{-1} fw d^{-1}; Table 1, considering an average earthworm biomass of 0.49 g for *A. caliginosa*) were similar to the reported rate of 1.5 mg CO_2 g^{-1} fw d^{-1} by Bayley et al. (2010) [54], and respiration rates for *A. tuberculata* (1.0–1.4 mg CO_2 g^{-1} fw d^{-1}; Table 1, average earthworm biomass of 1.06 for *A. tuberculata*) were within the range of 1.1–2.2 mg CO_2 g^{-1} fw d^{-1} reported by Phillipson and Bolton (1976) [4] for *Aporrectodea rosea* (an epi-endogeic species) and *Octolasium cyaneum* (an epi-*endo*-anecic or intermediate earthworm). This agreement with literature data add also support for the measurements obtained by calorimetry, because experiments in the calorimeter were carried out under the same environmental conditions as in the respirometer. Results from respirometry and isothermal calorimetry were consistent and suggest that soil compaction increases energy costs for burrowing. Our data indicate that the energy costs of burrowing are of the same order of magnitude or larger than basal metabolic costs of a “resting” earthworm, and hence constitute a significant part of the total energy budget.

4.1. Differences in respiration rates and energy costs between earthworm species

Basal respiration rates and basal energy costs were higher for *A. tuberculata* than for *A. caliginosa* (Table 1). This is primarily attributed to size differences, with *A. tuberculata* being about twice as large as

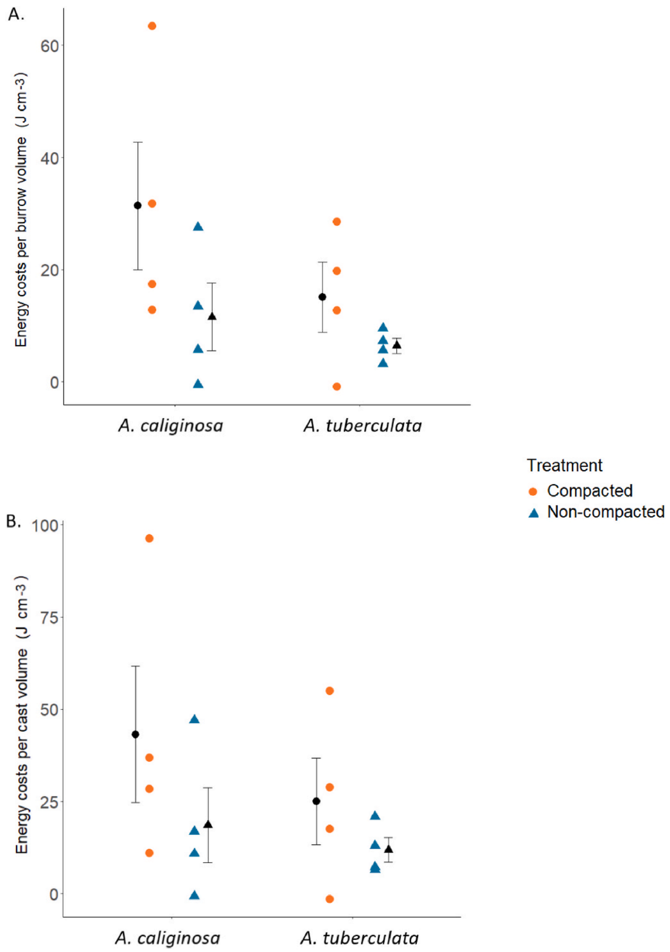

Fig. 4. **A)** Energy costs per burrow volume (J cm^{-3}), and **B)** energy costs per cast volume (J cm^{-3}) in compacted and non-compacted soil for *A. caliginosa* and *A. tuberculata*. The black circle (●) and triangle (▲) represent the mean values and the bars are standard errors of the means.

A. caliginosa. Our data do not provide a clear picture of the basal metabolism of the two species, as the specific basal respiration rates suggest higher values for *A. caliginosa*, while the specific basal energy costs (heat dissipation measurements) were similar for both species. We hypothesise that the differences in the ratio of energy consumption to respiration may indicate differences in metabolisms, as respiration measures processes resulting in CO_2 respiration whereas dissipated heat is the net outcome from aerobic and anaerobic processes.

Measurements suggest that *A. caliginosa* had higher energy costs and respiration rates associated to burrowing than *A. tuberculata*, and this trend was consistent for both respirometer and calorimeter measurements. This agrees with the fact that smaller earthworms commonly have higher respiration rates per unit of mass because of their higher specific surface area [4,54]. In our experiments, the energy costs associated with burrowing accounted for 40–70 % of the total energy costs, indicating that burrowing costs are a significant fraction of the total energy budget of earthworms.

4.2. *Impact of soil compaction on energy costs of burrowing*

Results from the isothermal calorimeter and respirometer measurements consistently indicated a similar trend, namely that respiration rates and energy costs of earthworms increase in compacted soil. Data from our experiments support previous studies that found that burrow volumes and lengths decrease in compacted soils [20,21,23,27,55]. Our results also suggest that energy costs per burrow volume increase in compacted soil, i.e. earthworms use more energy to create a certain volume of burrow in compacted soil, and this was also found in the data on respiration rates per burrow volume (Supplementary Material Fig. S2). This trend was consistent for both earthworm species, *A. caliginosa* and *A. tuberculata*, and supports results from our previous study where we estimated an increase in energy costs per burrow length with increasing soil mechanical resistance [23]. In agreement, Ruiz et al. (2015) [26] simulated that energy costs of bioturbation by roots and earthworms increase in compacted soil. A similar trend was also found for plant roots by Colombi et al. (2019) [56], who measured an increase in energy costs of root growth with higher penetration resistance. The similar response to compaction by roots and earthworms is interesting, as the penetration mechanism of plant roots is similar to that of earthworms that burrow [26,56].

There is a finite amount of energy available for earthworms in the form of litter at the soil surface or organic matter within the soil. Therefore, combined information on (i) burrowing rates (i.e., how much or how far earthworms burrow per unit of time) and (ii) the energy requirements for burrowing (i.e., how much energy earthworms need to create a burrow of a certain volume or length) are needed to estimate rates and amounts of earthworm burrowing for different soil conditions, and to better understand which factors constrain earthworm activity in a given soil. Such information will help to link soil ecosystem services supported by earthworm burrowing to land use and soil management that shape soil conditions and control the availability of food resources for earthworms.

4.3. *Estimation of energy costs for burrowing: methodological aspects*

To obtain the costs of burrowing, we subtracted the respiration or heat dissipation of earthworms on filter paper (without soil) and of soil (without earthworms) from the total respiration or heat dissipation from earthworms with soil, respectively. There are processes that we could not account for that may have influenced our results. For example, earthworms are known to activate soil microorganisms [57,58], and possibly the contribution from the soil (and the microorganisms in there) to total respiration and dissipated heat was underestimated in our samples with soil-only, which could lead to overestimated energy costs for burrowing. Scheu (1987) [59] quantified the microbial activity of burrow walls and cast of *A. caliginosa* for four weeks. It was found that during the first 48 h, the oxygen consumption from microbial activity was between 4 and 6 μL O_2 g^{-1} h^{-1} (burrow walls) and 7–12 μL O_2 g^{-1} h^{-1} (cast), which corresponds to energy costs of around 2–3 J g^{-1} d^{-1} (burrow walls) and 3.4–6 J g^{-1} d^{-1} (cast). These values account for 10–20 % of the total energy costs that we measured in the isothermal calorimeter (see Table 1). Thus, microorganisms in burrow walls and casts increase the overall energy consumption. However, their contribution is small compared to that of the burrowing earthworms.

Our data show that 42–73 % of the burrowing of *A. caliginosa* and *A. tuberculata* was done via ingestion (Table 3). It is known that the passage of material through the earthworm gut stimulates certain groups of microbes in the gut [45], and increased activity of gut microbiota may increase the overall heat production of earthworms. Consequently, when earthworms ingest more, some of the burrowing costs may be ascribed to higher heat production from gut microbiota. We believe this effect to be small, but it could be investigated in future research.

Measurements of earthworms without soil were done on moist filter paper, and this is a stressful situation for earthworms when they cannot find shelter. Hence, these measurements may overestimate basal respiration rates and basal energy requirements of earthworms “at rest”. If this was the case, our calculations underestimated the energy costs for burrowing. Yonemura et al. (2019) [41] made similar measurements with earthworms without soil and suggested that it is probable that earthworms moved in search of soil. Another probable effect that might have caused stress for earthworms was the temperature used for the experiments (20 °C). This is relatively high although not an unrealistic soil temperature. The temperature resulted from limitations in the respirometer device used in our study. Because we wanted to compare results from respirometry and calorimetry, the same temperature was chosen in both devices.

4.4. Potential and limitations of calorimetry to quantify energy costs of earthworm burrowing

The two sets of measurements in this study (using the two methods) were independent of each other but had the same experimental conditions (i.e., soil matric potential and temperature). We found a positive correlation between respiration rates and dissipated heat (Fig. 2), indicating a potential of using isothermal calorimetry for quantification of earthworm energy consumption. Further data are needed to confirm this correlation, and to test whether and how the relationship between respiration rate and dissipated heat depends on environmental conditions, earthworm species and earthworm activity level.

The largest limitation of using commercially available calorimeters in earthworm research is the size of the jars. The largest jars that fitted our calorimeter were relatively small (125 ml), and this small size is less suitable for larger species such as anecic earthworms. The size of the jars also restricts the duration of the measurements because oxygen depletes over time. For longer-term measurements, larger jars or regularly flushing the jars with oxygen during the measurements [54] would be necessary.

The use of calorimetry has plenty of potential for earthworm studies. Direct measurements of energy use are essential for quantifying calo-respirometric ratios, which provide information on metabolism and metabolic efficiency. We found a higher ratio of energy to respiration (J per unit CO_2 or unit O_2) than what is obtained when using the commonly applied oxyenergetic coefficient of 20.2 J ml^{-1} O_2 and respiratory quotient of 0.82 as suggested by Mehaan (2006) [36]. Further research is needed to quantify the oxyenergetic coefficients of earthworms and to explore the potential variation between species. Dissipated heat as measured in isothermal calorimeters covers both aerobic and anaerobic processes, in contrast to indirect estimation of energy costs from respirometry that relies on conversion coefficients based on aerobic respiration. Measurements in calorimeters could shed light on potential differences in metabolism between species and the impacts of soil conditions on metabolic reactions. Moreover, measurements of heat dissipation at high temporal resolution as is possible in calorimeters could reveal new insights into earthworm activity patterns that could contribute to understanding earthworm behaviour.

5. Conclusions

Soil compaction increases the mechanical resistance for burrowing by earthworms, and this study provided quantitative measurements of the energy costs associated with burrowing. We present the first encouraging results that show the potential of isothermal calorimetry as a method for the direct quantification of energy costs of earthworms. The method could be used in future studies to analyse the energy costs under different environmental conditions (e.g. temperature, moisture, compaction, soil, texture, food source, etc.). We provide data for earthworm energy costs of burrowing, for which so far only little information has been available. Information about costs for burrowing is necessary to better understand how soil properties and conditions affect earthworm burrowing dynamics and energy requirements, to develop quantitative links between land use and soil management (including undesired effects such as compaction) and soil functions and ecosystem services supported by earthworm activity.

CRediT authorship contribution statement

Elsa M. Arrázola-Vásquez: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mats Larsbo:** Writing – review & editing, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Yvan Capowiez:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Astrid Taylor:** Writing – review & editing, Conceptualization. **Anke M. Herrmann:** Writing – review & editing, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Thomas Keller:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejsobi.2024.103619.

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