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# How earthworms thrive and drive silicate rock weathering in an artificial organo-mineral system

Tullia Calogiuri[a,b,*], Iris Janssens[c], Alix Vidal[a], Jan Willem Van Groenigen[a], Tim Verdonck[d], Thomas Corbett[e], Jens Hartmann[f], Anna Neubeck[e], Harun Niron[g], Reinaldy P. Poetra[f], Lukas Rieder[f], Thomas Servotte[d], Abhijeet Singh[e], Michiel Van Tendeloo[g], Siegfried E. Vlaeminck[g], Sara Vicca[g,h], Mathilde Hagens[b]

[a] *Soil Biology Group, Wageningen University & Research, Wageningen, the Netherlands*
[b] *Soil Chemistry Group, Wageningen University & Research, Wageningen, the Netherlands*
[c] *IDLab—Department of Computer Science, University of Antwerp—imec, Antwerp, Belgium*
[d] *Department of Mathematics, University of Antwerp—imec, Antwerp, Belgium*
[e] *Department of Earth Sciences, Uppsala University, Uppsala, Sweden*
[f] *Institute for Geology, University of Hamburg, Hamburg, Germany*
[g] *Biobased Sustainability Engineering (SUSTAIN), University of Antwerp, Antwerp, Belgium*
[h] *Global Change Ecology Centre of Excellence, University of Antwerp, Antwerp, Belgium*

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## ABSTRACT

To slow the rise in atmospheric carbon dioxide concentrations, Enhanced Silicate Weathering is emerging as a potentially significant Carbon Dioxide Removal technology. However, the biotic controls on rock weathering are not well understood, particularly for key soil faunal groups such as earthworms. Earthworms have shown to possibly enhance weathering, highlighting their potential to be introduced in controlled or engineered settings, such as reactors, to increase carbon sequestration. Here, we determined the potential for earthworms to thrive and to increase weathering rates in an artificial organo-mineral system simulating a bioreactor. We used two earthworm species (*Aporrectodea caliginosa* [Savigny] and *Allolobophora chlorotica* [Savigny]) at four densities (10, 20, 25 and 30 earthworms $kg^{-1}$ organo-mineral mixture), four silicate rock types (two basanites, dunite and diabase) of two to three grain sizes (d50 between 0.026 and 1.536 mm), two sources of organic materials (straw and co-digestate), two amounts of biochar (0 and 100 g $kg^{-1}$ organo-mineral mixture) and/or enzyme additions (laccase, urease and carbonic anhydrase at 20, 177 and 1955 units $kg^{-1}$ organo-mineral mixture, respectively), three water irrigation rates (125, 250 and 375 mL $day^{-1}$ $kg^{-1}$ organo-mineral mixture) and three watering frequencies (one, two and five times $day^{-1}$). The experiment was conducted in eight rounds, each one lasting eight weeks, yielding data for a total of 323 experimental units. We measured earthworm survival and activity, as well as several commonly used weathering indicators in the organo-mineral mixture and in the leachate, as total alkalinity, inorganic carbon, pH, electrical conductivity and major cations. Using random forest regression, we found that earthworm survival and activity mainly depended on variables influencing the structure and drainage potential of the organo-mineral mixture, such as the presence of straw and increasing percentages of coarse grain sizes. Furthermore, we concluded that the effect of earthworms on weathering indicators depended on whether they survived or died by the end of the experimental period. Surviving earthworms had a neutral or negative effect on weathering indicators, likely because the experimental duration was too short to detect an increase in inorganic carbon, or because there was an increase in organic rather than inorganic carbon in the organo-mineral mixture. In contrast, dead earthworms enhanced almost all weathering indicators considered, suggesting that microbial processes associated with decomposing earthworm bodies may play a role in enhancing weathering. Our results also emphasize that the role of earthworms in Enhanced Silicate Weathering within bioreactors might be overestimated if weathering indicators exclusively rely on changes in mineralogy and ions release to quantify earthworm effects on carbon sequestration through weathering.

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[*] Corresponding author. Wageningen University & Research, Droevendaalsesteeg 3, 6708 PB, Wageningen, the Netherlands.
*E-mail address:* tullia.calogiuri@wur.nl (T. Calogiuri).

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## 1. Introduction

If global temperature increases are to be kept below 1.5 °C to achieve the climate target set by the Paris Agreement, Carbon Dioxide Removal (CDR) technologies are needed to compensate for anthropogenic emissions, capturing and storing atmospheric carbon dioxide ($CO_2$) (Strefler et al., 2021; Pörtner et al., 2022). Enhanced Silicate Weathering (ESW) has been proposed as a CDR technology with a high potential (Hartmann et al., 2013; Strefler et al., 2018). This technology is based on the natural process of rock weathering, which is an integral part of the global carbon cycle and removes about 1.1 Gt $CO_2$ $y^{-1}$ from the atmosphere in the form of inorganic carbon (IC) (Hartmann et al., 2009; Strefler et al., 2021). ESW aims at accelerating the natural weathering process by crushing rocks rich in silicate minerals up to the μm grain size and spreading these powdered rocks on soils which are naturally $CO_2$-rich (Hartmann et al., 2013). During natural weathering, $CO_2$ dissolved in water reacts with the silicate minerals, and the dissolution products can either precipitate in the soil or be transported to the ocean, where they can be stored as carbonate minerals in geologic formations over at least thousands of years (Goudie and Viles, 2012; Strefler et al., 2018).

Most studies on ESW rates have focused on the controlling abiotic factors. These studies observed high weathering rates when using fine grain sizes and specific silicate mineral types, such as olivine, and under conditions of high acidity, $CO_2$ concentrations, temperature and water flow (Kump et al., 2000; Romero-Mujalli et al., 2019; West et al., 2005; White and Buss, 2014). Biotic factors can also accelerate mineral weathering rates, but their role in ESW is understudied (Vicca et al., 2022).

Earthworms are among the most important soil ecosystem engineers and therefore of primary interest with respect to biotic controls on ESW (Vidal et al., 2023). Different mechanisms have been proposed through which earthworms could enhance mineral weathering. By respiring $CO_2$ and increasing its release from faster decomposition of plant residues, earthworms could contribute to mineral weathering due to enhanced acidification (Lubbers et al., 2013; Schwartzman, 2015). Earthworms could also increase the available reactive surface area of the minerals through grinding in their gizzard during particle ingestion (Suzuki et al., 2003). Microbes dwelling in earthworm intestines could further attack these particles and enhance weathering through lowering pH and/or excreting chelators and enzymes (Needham et al., 2004; Carpenter et al., 2007, 2008; Uroz et al., 2009; Liu et al., 2011, 2018; Georgiadis et al., 2019; Jafari et al., 2021, 2022). Besides processes inside the earthworm, the burrowing and casting activities of earthworms are known to enhance microbial activity in the earthworm-affected environment, which could further stimulate mineral weathering (Carpenter et al., 2007; Liu et al., 2011). Finally, earthworms also create macropores through their burrowing activity, which results in an increase of the rock-water contact area and avoids supersaturation of pore water with respect to secondary mineral phases (Schwartzman, 2015).

Several studies provide evidence that earthworms can enhance mineral weathering, mostly focusing on the effect of earthworms on changes in mineralogy or nutrient release from minerals (Suzuki et al., 2003; Carpenter et al., 2007; Bityutskii et al., 2016; Jafari et al., 2021, 2022). Recently, Vienne et al. (2024) investigated the effect of earthworms on commonly used weathering indicators including total alkalinity (TA), inorganic carbon (IC), pH, and major cations in a mesocosm experiment mimicking field conditions by mixing basalt with soil. They found evidence of for earthworm-enhanced weathering under these non-optimized conditions. Knowing that selected abiotic factors accelerate weathering and that earthworms could further enhance this process, this creates the potential for the introduction of earthworms in more controlled settings aimed at $CO_2$ capture through enhanced weathering, such as bioreactors. To date, the potential of using macrofauna to enhance weathering for carbon capture within bioreactors remains unknown.

Here, we present the first exploratory study focusing on earthworm effects on enhanced weathering in controlled small-scale reactors in which multiple relevant abiotic factors were manipulated. One condition for reaching the maximum potential of earthworm-enhanced weathering in such an artificial system is that earthworms are able to thrive. To determine their optimal living conditions, we investigated earthworm survival and activity for two different earthworm species under a range of abiotic experimental conditions. These included combinations of silicate rock powders and grain sizes, organic materials, biochar and enzyme additions, water irrigation rates and watering frequencies. Similar to Li et al. (2021), Nathwani et al. (2022) and Siqueira et al. (2024) we combined random forest modelling with model explainability methods, to learn how the living conditions impacted earthworm survival and activity. To determine the effect of earthworms on mineral weathering in this artificial system we measured various routinely used indicators to assess weathering rates and carbon (C) sequestration, including TA, IC, pH, electrical conductivity (EC), and major cations (Hartmann et al., 2013; Anda et al., 2015; Amann and Hartmann, 2022; Amann et al., 2022; Vienne et al., 2022; Campbell et al., 2023).

## 2. Materials and methods

### 2.1. Selection and characterisation of materials

To reach a higher weathering rate, we selected four rock powders: two basanites (RPBL, Germany), to which we refer to as basanite 1 and basanite 2 as they differed in their mineralogical composition, dunite (Sibelco, Norway) and diabase (Schicker Mineral, Germany). These rocks were chosen because they are mainly composed of relatively fast-weathering silicate minerals such as forsterite and nepheline (Table S1; Drever, 1997) and because of their broad commercial availability. These rock powders presented two or three main classes of grain sizes, fine, medium and coarse, depending on the rock type, as one of the abiotic factors determining mineral weathering rates is the grain size (Strefler et al., 2018). Despite hypotheses suggesting that finer grain sizes might lead to higher weathering rates due to a higher available surface area, the grain size effect may be limited, as observed by Amann et al. (2022). This suggests that an optimal combination between fine and coarse grain sizes should be found to increase weathering rates and favour an optimal water flow and rock-water contact. We determined the initial particle size distribution (PSD) of the rock powders through laser diffraction (particle-sizer Sympathec HELOS/KF Magic) (Table 1 and Figs. S1 and S2) and their initial elemental composition through X-ray fluorescence (XRF) (Panalytical Magix Pro) (Table 1 and S2). The mineralogical composition of the rock powders was characterised through X-ray diffraction (XRD) (STOE X-ray diffractometer) (Table S1) and the approximate quantification of the minerals present in the rocks was done using an approach from CIPW norm following Cross et al. (1902) (Table 2). The XRD analysis was done to check the presence of a possible mineral, therefore the analysis was qualitative as the quantification of their fraction was not available. The rough estimation of the minerals' fraction was calculated using the CIPW norm according to the elemental composition of the rocks, as presented in Table 1. The initial solid inorganic carbon (SIC) content of the rock powders was determined after removal of solid organic carbon (SOC) through loss on ignition (LOI) (Koorneef et al., 2023). Samples were combusted for 3 h at 550 °C followed by IC analysis on the ash using an elemental analyser (Flash, 2000 CN Soil Analyser, Interscience, Louvain-la-Neuve, Belgium) (Table 1).

Since the initial organic carbon content of the rocks was very low and nitrogen was undetected (below detection limit of 0.1 mg $g^{-1}$), wheat straw (Pets Place, The Netherlands) and co-digestate of pig manure and biowaste (Groene Mineralen Centrale, The Netherlands) were selected as a food source for earthworms. Besides, organic residues decomposition leads to the release of $CO_2$, which can further accelerate weathering (Ajwa and Tabatabai, 1994; Amann et al., 2022). Biochar (Ithaka Institute for Carbon Strategies, Germany) from feedstock was also added

**Table 1**

d10, d50 and d90 of the particle size distribution (PSD), elemental composition, and solid inorganic carbon (SIC) content of basanite 1, basanite 2, dunite and diabase. Values for d10, d50 and d90 are expressed in mm, values for elements are expressed as % oxides and for SIC and LOI are expressed as %. Values for elements and SIC refer to mean and standard deviation (n = 3). Values for d10, d50 and d90 refer to a single sample. Repeated measurements were not done for the PDS of the rock powders because replicated measurements on test samples did not differ between each other. d10, d50 and d90 values for coarse basanite 1 are missing as this rock powder is not suitable for the Laser Particle Analyser. The particle size range as given by the supplier was 1–3 mm. When manually sieved, the particle size range of basanite 1 fell between 1 and 4 mm, with only 1.02 % of the total sample lying between 3.15 and 4 mm sieve.

| Rock powder | d10 | d50 | d90 | CaO | MgO | MnO | $SiO_2$ | $Fe_2O_3$ | $Na_2O$ | $K_2O$ | $P_2O_5$ | $Al_2O_3$ | $TiO_2$ | SIC | LOI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Basanite 1: fine | 0.003 | 0.026 | 0.096 | 10.41 ± 0.02 | 11.4 ± 0.31 | 0.17 ± 0 | 43.87 ± 0.55 | 11.66 ± 0.18 | 2.26 ± 0.01 | 1.01 ± 0.06 | 0.62 ± 0 | 12.13 ± 0.18 | 2.15 ± 0.06 | 0.134 ± 0.01 | 0.70 ± 0.45 |
| Basanite 1: coarse | – | – | – | 10.41 ± 0.02 | 11.4 ± 0.31 | 0.17 ± 0 | 43.87 ± 0.55 | 11.66 ± 0.18 | 2.26 ± 0.01 | 1.01 ± 0.06 | 0.62 ± 0 | 12.13 ± 0.18 | 2.15 ± 0.06 | 0.068 ± 0.01 | 1.37 ± 0.09 |
| Basanite 2: fine | 0.008 | 0.035 | 0.073 | 12.6 ± 1 | 8.68 ± 0.12 | 0.17 ± 0.01 | 43.44 ± 0.27 | 11.19 ± 0.26 | 2.99 ± 0.08 | 3.29 ± 0.09 | 0.53 ± 0.07 | 13.79 ± 0.25 | 2.58 ± 0.33 | 0.009 ± 0.01 | 0.23 ± 0.10 |
| Basanite 2: coarse | 0.924 | 1.536 | 1.340 | 12.6 ± 1 | 8.68 ± 0.12 | 0.17 ± 0.01 | 43.44 ± 0.27 | 11.19 ± 0.26 | 2.99 ± 0.08 | 3.29 ± 0.09 | 0.53 ± 0.07 | 13.79 ± 0.25 | 2.58 ± 0.33 | 0.006 ± 0.01 | 0.14 ± 0.12 |
| Dunite: fine | 0.004 | 0.029 | 0.099 | 0.35 ± 0.19 | 47.87 ± 0.24 | 0.09 ± 0 | 41.55 ± 0.23 | 7.26 ± 0 | 0.03 ± 0 | 0.03 ± 0.01 | 0.01 ± 0 | 0.78 ± 0.01 | 0.02 ± 0.01 | 0.026 ± 0.01 | 1.80 ± 0.16 |
| Dunite: medium | 0.135 | 0.209 | 0.260 | 0.35 ± 0.19 | 47.87 ± 0.24 | 0.09 ± 0 | 41.55 ± 0.23 | 7.26 ± 0 | 0.03 ± 0 | 0.03 ± 0.01 | 0.01 ± 0 | 0.78 ± 0.01 | 0.02 ± 0.01 | 0.005 ± 0.01 | 0.31 ± 0.21 |
| Dunite: coarse | 0.769 | 1.314 | 1.740 | 0.35 ± 0.19 | 47.87 ± 0.24 | 0.09 ± 0 | 41.55 ± 0.23 | 7.26 ± 0 | 0.03 ± 0 | 0.03 ± 0.01 | 0.01 ± 0 | 0.78 ± 0.01 | 0.02 ± 0.01 | 0.008 ± 0.004 | 0.45 ± 0.18 |
| Diabase: fine | 0.005 | 0.024 | 0.066 | 14.29 ± 0.2 | 4.86 ± 0.12 | 0.14 ± 0.01 | 35.93 ± 0.73 | 13.31 ± 0.13 | 1.81 ± 0.03 | 1.61 ± 0.11 | 0.41 ± 0.01 | 11.83 ± 0.13 | 2.77 ± 0.09 | 2.419 ± 0.03 | 2.52 ± 0.32 |
| Diabase: coarse | 0.326 | 1.144 | 1.793 | 14.29 ± 0.2 | 4.86 ± 0.12 | 0.14 ± 0.01 | 35.93 ± 0.73 | 13.31 ± 0.13 | 1.81 ± 0.03 | 1.61 ± 0.11 | 0.41 ± 0.01 | 11.83 ± 0.13 | 2.77 ± 0.09 | 1.717 ± 0.11 | 1.93 ± 0.50 |

**Table 2**

Major contributions to the normative composition of the rock flours according to their elemental compositions calculated using the CIPW norm approach from Cross et al. (1902). Normative composition of diabase might be biased because of the presence of altered minerals, i.e., calcite. Asterisks indicate the minerals' fraction that was not detected with X-ray diffraction (XRD).

| Rock flours | Normative Composition | Percentage (%) |
|---|---|---|
| Basanite 1 | Anorthite | 19.97 |
| | Albite | 19.12 |
| | Diopside* | 16.22 |
| | Hematite* | 11.66 |
| | Olivine* | 11.09 |
| | Orthoclase* | 5.97 |
| | Hypersthene* | 5.05 |
| | Titanite* | 4.81 |
| | Apatite* | 1.43 |
| | Ilmenite* | 0.36 |
| Basanite 2 | Diopside | 30.55 |
| | Orthoclase* | 15.82 |
| | Anorthite* | 14.49 |
| | Nepheline | 13.71 |
| | Hematite* | 11.19 |
| | Olivine* | 5.23 |
| | Perosvskite* | 4.07 |
| | Leucite* | 1.32 |
| | Ilmenite* | 0.36 |
| | Apatite* | 0.16 |
| Dunite | Olivine* | 90.21 |
| | Hematite* | 7.1 |
| | Anorthite* | 1.67 |
| | Albite* | 0.25 |
| | Magnetite* | 0.24 |
| | Orthoclase* | 0.18 |
| | Corundum* | 0.09 |
| | Ilmenite* | 0.04 |
| | Apatite* | 0.02 |
| Diabase | Diopside* | 26.11 |
| | Anorthite* | 19.4 |
| | Hematite* | 13.31 |
| | Calcite* | 13.04 |
| | Orthoclase* | 9.51 |
| | Nepheline* | 5.87 |
| | Albite | 4.48 |
| | Perovskite | 4.45 |
| | Wollastonite | 2.59 |
| | Apatite | 0.94 |
| | Ilmenite | 0.30 |

to columns to reduce metal availability (Park et al., 2011) released from the rocks (Table S2). The elemental composition of all organic materials was determined using ICP-OES (Thermo iCAP 6500 dual view) after digestion with nitric- and hydrochloric acid and being heated in a microwave, adapted from Novozamsky et al. (1996). Total carbon (C) and nitrogen (N) contents were determined using an elemental analyser (FlashSmart, Thermo Fisher Scientific, USA) (Table 3).

Two endogeic earthworm species, *Aporrectodea caliginosa* [Savigny] and *Allolobophora chlorotica* [Savigny], were selected for this study because of their high abundance in the Netherlands and because of their feeding habit, primarily feeding on soil particles, and associated organic matter (Bouché, 1977). Due to their feeding activity, these earthworm species were expected to have a greater potential in enhancing weathering mostly through the physical and chemical processes happening within their bodies (Suzuki et al., 2003; Carpenter et al., 2007, 2008; Uroz et al., 2009; Liu et al., 2011, 2018; Georgiadis et al., 2019). Both juveniles and adults earthworms were collected at the beginning of each round of experiments from the park De Blauwe Bergen in Wageningen, The Netherlands (51°58′51.8"N 5°39′38.0"E). The differentiation between juveniles and adults within the same species was based on the absence or presence of the clitellum in juveniles and adults, respectively. The identification of juveniles between different species was made possible by carefully selecting the collection locations. Specifically, juveniles of *A. chlorotica* were collected from a designated area within the

**Table 3**

Elemental composition of straw, co-digestate and biochar expressed in mg $g^{-1}$. Values represent mean and standard deviation (n = 4).

| | Elements | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Organic matter type | C | N | P | K | Ca | Mg | Zn | Fe |
| Wheat straw | 443.64 ± 9.83 | 5.16 ± 0.97 | 0.47 ± 0.01 | 8.00 ± 0.08 | 2.92 ± 0.06 | 0.46 ± 0.01 | 0.004 ± 0.001 | 0.11 ± 0.02 |
| Co-digestate | 442.24 ± 6.90 | 8.76 ± 0.53 | 4.72 ± 0.38 | 2.75 ± 0.06 | 12.38 ± 1.44 | 1.31 ± 0.03 | 0.14 ± 0.01 | 1.18 ± 0.09 |
| Biochar | 782.79 ± 7.24 | 5.53 ± 0.26 | 1.41 ± 0.03 | 6.58 ± 0.05 | 19.58 ± 1.20 | 1.53 ± 0.20 | 0.09 ± 0.002 | 0.33 ± 0.03 |

park De Blauwe Bergen, where this species is exclusively found. For *A. caliginosa*, juveniles were collected from another area in the park where the only other species present was the epigeic species *Lumbricus rubellus*. In this case, identification between juveniles of the two species was based on pigmentation.

Three enzymes, carbonic anhydrase, urease and laccase (all obtained from Sigma-Aldrich Chemie GmbH), were added to the columns as a potential stimulus for mineral dissolution (Sun et al., 2013), but their effect on weathering is not investigated in this study. Natural groundwater was used for irrigation, the composition of which is given in Table S3. Natural groundwater was continuously supplied during the experimental runs, rather than being collected and stored in advance. This was achieved through a tap connected to the irrigation system, ensuring a steady and ongoing replenishment of water. Three water irrigation rates (125, 250 and 375 mL $day^{-1}$ $kg^{-1}$ organo-mineral mixture), and three watering frequencies (one, two and five times $day^{-1}$) were used. We selected different irrigation rate regimes and frequencies because water volume and residence time are known for stimulating weathering by increasing the physical degradation of the rocks and by preventing the solute concentrations of pore waters in which rocks react to reach saturation (West et al., 2005; White and Buss, 2014).

### 2.2. Experimental set-up

Eight rounds of eight-week column experiments were carried out in a climate chamber at 25 °C, yielding a total of 323 columns. We chose 25 °C as our experimental temperature to enhance weathering rates, which are known to increase with higher temperatures (Kump et al., 2000; Li et al., 2016), while simultaneously ensuring earthworms' survival in the system, which generally prefer lower temperatures of around 15 °C (Lowe and Butt, 2005; Edwards and Arancon, 2022). Across the different experimental rounds, we had different numbers of columns per round, as the experiments were part of a larger investigation and a selection of the treatments was made to fulfil the objectives of this study. The experimental length of eight weeks per round was chosen because the aim was to simulate a 2-month processing of the organo-mineral mixture in a bioreactor where earthworms were introduced. All treatments were present as unique combinations, so that no treatment was replicated over the eight experimental rounds. This was done to cover the widest possible range of combinations between the different factors based on expert knowledge in the given number of experimental rounds.

At the beginning of each round of experiments, columns (diameter 7 cm, height 15 cm) were filled with 400 g (dry weight) of rock powder. The microcosms were not only filled with a single rock type and a single grain size, but also included mixtures of rock types and/or of grain sizes at different percentages ranging from 0% to 100%. To the 400g of rock powder, 10g of straw or co-digestate was added and homogenised. Selected treatments received the addition of 40g of biochar. To ensure the same starting conditions, water was then added to reach 80% of the water holding capacity (WHC) for all treatments. WHC was determined as described in Calogiuri et al. (2023). In short, rock flours were first dried at 105 °C. Afterwards, the dried material was placed in a bowl and water was added little by little until water could be seen in the openings of the rock flour. After recording the weight before and after water addition, the WHC was then calculated as:

$$WHC = \frac{\left(weight_{dry\ rock\ flour}(g) - weight_{wet\ rock\ flour}(g)\right)}{weight_{dry\ rock\ flour}(g)}\ \text{x}\ 100 \tag{1}$$

At the start of the experiments, juvenile and mature earthworms were placed on the surface of the columns after starving for two days and recording their weight, following the method of Dalby et al. (1996). Individual fresh weight was 0.26 ± 0.13 g and 0.26 ± 0.08 g for *A. caliginosa* and *A. chlorotica*, respectively. Earthworms were introduced at four densities (10, 20, 25 and 30 earthworms $kg^{-1}$ organo-mineral mixture), and were left to acclimatise to their new environment for 24 h before the start of irrigation. We chose these earthworm densities because we aimed to mimic conditions in a reactor in which the optimal earthworm density for enhancing weathering still needed to be determined. It has to be noted that these densities do not reflect realistic densities under natural conditions. At the end of each cycle of experiments, earthworms were counted, and their activity was measured following the method of Garamszegi et al. (2024). Shortly, alive and moving earthworms were introduced in a cylinder filled with a sugar solution of a density of 1.08 g $cm^{-3}$. Earthworms that sunk at the bottom of the cylinder were considered as active as they had a higher density compared to the sugar solution, due to the ingestion and presence of mineral particles in their gut. Earthworms that floated on the surface of the solution were considered inactive as their density was the same as the sugar solution, implying that their gut was empty as they have not been feeding. Further, earthworm weight was recorded after emptying their guts for two days. Afterwards, earthworm survival rate was calculated as:

$$Survival = \frac{number\ of\ surviving\ earthworms}{number\ of\ introduced\ earthworms}\ x\ 100 \tag{2}$$

while earthworm activity rate was calculated as:

$$Activity = \frac{number\ of\ active\ earthworms}{number\ of\ surviving\ earthworms}\ x\ 100 \tag{3}$$

For selected treatments, the three different enzymes were added together at the beginning and in the middle of the experimental period at doses of 20, 177 and 1955 units $kg^{-1}$ organo-mineral mixture for laccase, urease, and carbonic anhydrase, respectively. Laccase was dosed based on concentrations found in natural quartz weathering (Kirtzel et al., 2020), urease concentrations were similar to those utilised in the enzymatic modification of porous media via $CaCO_3$ precipitation (Nemati and Voordouw, 2003), and carbonic anhydrase concentrations were at the upper limits of those in natural soils (Sauze et al., 2018). After earthworm addition and the first enzymatic inoculation, columns were watered daily via a downflow automated irrigation system, and connected through pipes to jerrycans placed in a fridge in which leachate was collected over the eight weeks. A detailed description of the experimental setup is given in Calogiuri et al. (2023).

### 2.3. Chemical analyses

At the end of each experimental round, chemical analyses were carried out for both the organo-mineral mixture and the leachate. Organo-mineral samples of each microcosm were first dried at 40 °C for 5 days and the coning-quartering method (Campos-M and Campos-C, 2017) was used for sample collection to ensure homogeneity. Samples were subsequently colloid grinded and SIC (%) was determined as described in section 2.1. The change of SIC over time (ΔSIC) was calculated by subtracting the initial SIC of the rock powders from the final SIC measured in the organo-mineral mixture. Initial and final SIC were corrected considering the weight loss after LOI.

The collected leachate was filtered through a 0.45 μm filter. Total dissolved carbon (TDC, mg $L^{-1}$) and dissolved inorganic carbon (DIC, mg $L^{-1}$) content were determined using a TOC analyser (Autosampler LAS-160 Skalar), from which we calculated dissolved organic carbon (DOC) by difference. The filtered leachate was also analysed for TA (mmol $L^{-1}$) using a Metrohm Titrando (600 series) (Amann et al., 2022) and for pH (expressed as [H+]) and EC (μS $cm^{-1}$) using a multi-parameter portable meter (WTW Multiline® Multi 2630 IDS). We further measured ions as calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P) and iron (Fe) using ICP-MS (Thermo Element 2, Thermo Fisher Scientific, USA) or ICP-OES (Thermo iCAP 6500 dual view) depending on ion concentrations. Silicon (Si) was measured using a spectrophotometer (Hach Lange spectrophotometer LPG 429.99.00001) at a wavelength of 810 nm based on the molybdate-blue method, according to Hansen and Koroleff (1999). Samples for ion analyses were not acidified as initial testing showed no differences in ion concentrations between acidified and non-acidified samples. Chemical analyses were generally carried out within one week from the sampling. Details on accuracy, precision and detection limits for the different chemical analyses are reported in Tables S4, S5, S6 and S7. Results for DIC and DOC were first converted to mmol $L^{-1}$, and afterwards DIC, DOC, TA and major cations were converted from mmol $L^{-1}$ to mmol using the total amount of leachate collected by the end of the eight-week experimental period. Total inorganic carbon (TIC) gain was calculated by summing ΔSIC in the organo-mineral mixture and the cumulative DIC in the leachate.

## 2.4. Data analyses

Out of a total of 323 columns with different combinations, 199 columns in which earthworms were introduced were used for the first objective of this study, i.e. to determine the optimal living conditions for earthworms in the organo-mineral system. A total of 71 paired couples, equal to 142 treatments, were used to assess the second objective, which was to determine the effect of earthworms on weathering indicators.

### 2.4.1. Optimal conditions for earthworm survival and activity

To address the first objective of this paper, i.e. to determine the optimal living conditions for earthworms in the system, we posed two binary classification problems; to predict, based on the system conditions.

i. whether earthworm survival was above or below 50%;
ii. whether earthworm activity was above or below 50%.

Therefore, two random forest classifiers were trained using Python (version 3.10.12). For the first classification problem, all 199 treatments with earthworms were taken into account. For the second classification problem, treatments where no earthworms survived were excluded from the analysis, leading to a total of 164 treatments. The features considered in the models represent the system conditions and the characteristics of the added earthworms based on density and species, and are listed in Table 4.

To be able to perform model training, hyperparameter tuning and model evaluation on the dataset without obtaining optimistically biased results (Stone, 1974), a nested cross validation approach was adopted for model training and evaluation. This approach involved two layers of cross validation: an inner 10-fold cross validation loop, used for training and hyperparameter tuning, and an outer 10-fold cross validation loop, used to test the model performance and to remove bias due to the randomness of the train-test split. In essence, the data was split into 10 datasets using stratified sampling, with one set serving as the test set and the other nine as the training set of the outer cross validation loop. The training set was further split into 10 sets for the inner cross validation loop, in which the random forest classifiers were trained and optimal hyperparameters were selected using a gridsearch by optimizing the balanced accuracy (Eq. (4)). The hyperparameters that were explored in the gridsearch were max_depth (2,3,4,5,6), n_estimators (25,50,100, 200,400,800), min_samples_split (3,4,5,6) and min_samples_leaf (2,3,4, 5). The trained model was then tested using the test set of the outer cross validation loop. This process was repeated 10 times, each time with another set as the test set of the outer cross validation loop, yielding a total of 10 trained models for both earthworm survival and activity.

The classification performance of the models was evaluated by averaging the performances of the 10 random forest classifiers of the outer cross validation loop. The considered performance metrics were the area under the receiver operating characteristic (ROC-AUC), the Brier Score and the balanced accuracy. The ROC-AUC is defined as the area under the curve formed by plotting the true positive rate (TPR) against the false positive rate (FPR) for different classification thresholds, and was calculated using the “roc” function from the “pROC” package (Robin et al., 2011). The Brier score measures the accuracy of a predicted probability and was calculated as:

**Table 4**
List of the features, their description and possible values considered in the random forest model to determine earthworm best living conditions in the system.

| Model feature | Description | Possible values |
|---|---|---|
| Water irrigation rate | Amount of water used for daily irrigation | [125, 250 and 375 mL $day^{-1}$ $kg^{-1}$ organo-mineral mixture] |
| Watering frequency | Frequency at which irrigation water was given | [1, 2, 5 times $day^{-1}$] |
| Fine basanite 1 % | Proportion of basanite 1 with fine grain size w.r.t. rock mixture | [0–100%] |
| Coarse basanite 1 % | Proportion of basanite 1 with coarse grain size w.r.t. rock mixture | [0–100%] |
| Fine basanite 2 % | Proportion of basanite 2 with fine grain size w.r.t. rock mixture | [0–100%] |
| Coarse basanite 2 % | Proportion of basanite 2 with coarse grain size w.r.t. rock mixture | [0–100%] |
| Fine dunite % | Proportion of dunite with fine grain size w.r.t. rock mixture | [0–100%] |
| Medium dunite % | Proportion of dunite with medium grain size w.r.t. rock mixture | [0–100%] |
| Coarse dunite % | Proportion of dunite with coarse grain size w.r.t. rock mixture | [0–100%] |
| Fine diabase % | Proportion of diabase with fine grain size w.r.t. rock mixture | [0–100%] |
| Coarse diabase % | Proportion of diabase with coarse grain size w.r.t. rock mixture | [0–100%] |
| Biochar mass | Amount of biochar added | [0, 100 g $kg^{-1}$ organo-mineral mixture] |
| Organic matter type | Type of organic matter added | [Straw, co-digestate] |
| Enzyme addition | Whether the mix of enzymes (laccase, urease, carbonic anhydrase) was added | [yes, no] |
| Earthworm number | Number of earthworms | [10, 20, 25 and 30 earthworms $kg^{-1}$ organo-mineral mixture] |
| Earthworm species | Ratio of *A. chlorotica* earthworms w.r.t. total number of earthworms, i.e. 1 means 100% of the earthworms were *A. chlorotica*, 0 means 100% of the earthworms were *A. caliginosa*, 0.5 means 50% of the earthworms were belonging to each of the two species | [0, 0.5, 1] |

$$Brier\ score = \frac{1}{n}\sum_{i=1}^{n}(p_i - z_i)^2 \tag{4}$$

where n is the number of observations, $p_i$ the predicted probability of outcome 1 of observation i, and $z_i$ the actual binary outcome of observation i. The balanced accuracy is the arithmetic mean of sensitivity and specificity and gives a more realistic picture of the model performance than the accuracy in case of imbalanced classes. The balanced accuracy was calculated as:

$$balanced\ accuracy = 1 \Big/ 2\left(sensitivity\ +\ specificity\right)$$
$$= \frac{1}{2}\left(\frac{true\ positives}{true\ positives + false\ negatives} + \frac{true\ negatives}{true\ negatives + false\ positives}\right) \tag{5}$$

Explainability was added ad hoc to the models by calculating the feature importance of each model in the outer cross validation loop, based on the mean decrease in impurity. These importances represent how much each feature contributes on average to the prediction of earthworm survival and activity. Partial dependence plots (PDPs) of the four features with the highest feature importance were created to show the dependence between the target and the selected feature and thereby determine how the important features influence earthworm survival and activity. Model training, computing of feature importances and of partial dependences were done using Python's "sklearn" package (version 1.3.2).

### 2.4.2. Earthworm effect on mineral weathering indicators

The second objective of this study was to determine the effect of earthworms on weathering indicators, for which we used R (version 4.3.1). We first tested all parameters separately for normality through Shapiro-Wilk test, histograms and Q-Q plots. As data was not normally distributed, we applied the non-parametrical Wilcoxon test from the "stats" package (R Core Team, 2013) to the 71 paired observations which maintained the same conditions but differed only in whether earthworms were added or not. Further, we divided paired observations into two smaller datasets according to earthworm survival rate, above 75%, for a total of 42 couples of points, and below 25%, for a total of 22 couples of points. This was done to differentiate possible effects on weathering indicators derived from earthworms that survived (survival rate above 75%) and earthworms that died (survival rate below 25%) by the end of the experimental period. We expected decomposition of earthworm bodies to have a different effect on the selected indicators compared to the activity of surviving earthworms (Sun and Ge, 2021; Trap et al., 2021; Lin et al., 2022). Each weathering indicator was tested individually and the level of significance was set at $p < 0.05$. For the indicators of each dataset which showed a level of significance below 0.05, we tested for the effect size (r) using the "wilcox_effsize" function from the "rstatix" package (Kassambara, 2023) to measure the strength of the observed effect. The effect size was considered null for $r < 0.1$, small for $0.1 < r < 0.3$, medium for $0.3 < r < 0.5$ and large for $r > 0.5$ (Kassambara, 2023).

## 3. Results

### 3.1. Optimal conditions for earthworm survival and activity

Out of the 199 columns considered, 62% had an earthworm survival rate above 50%. In the columns where at least one earthworm survived, 43% had an earthworm activity rate of above 50%. The resulting performance metrics of the survival and accuracy random forest models are shown in Table 5. Earthworm survival proved to have the best predictive performance, while the model predicting earthworm activity had lower performance metrics. However, the latter model still significantly outperformed the naive prediction, i.e. predicting the majority class of the training sets, which has a balanced accuracy of 0.5.

**Table 5**
Classification performance of the random forest models predicting earthworm survival and activity. Presented values are the mean and standard deviation of the performance metrics of the 10 random forest models in the outer loop of the nested cross validation.

| Performance metric | Survival model | Activity model |
|---|---|---|
| ROC AUC | 0.72 ± 0.12 | 0.62 ± 0.14 |
| Brier score | 0.20 ± 0.03 | 0.23 ± 0.03 |
| Balanced accuracy | 0.66 ± 0.09 | 0.59 ± 0.07 |

The prediction of earthworm survival and activity was partly driven by different features (Figs. 1 and 2). The type of organic matter contributed most to the prediction of earthworm survival (Fig. 1a). This was followed, in order of decreasing importance, by the percentage of fine dunite, the percentage of coarse basalt and the earthworm species (Fig. 1a). Specifically, the PDPs show that straw led to higher probabilities of survival compared to digestate (Fig. 2a). Furthermore, increasing the percentages of dunite with fine grain sizes tended to decrease the probability of a high earthworm survival, while increasing percentages of basalt with coarse grain sizes led to the opposite effect (Fig. 2a). Regarding earthworm species, *A. caliginosa* showed a slightly higher probability to survive than *A. chlorotica* and a combination of the two (Fig. 2a).

The main driver of earthworm activity was the percentage of fine basalt (Fig. 1b), with PDPs indicating that high percentages led to lower probabilities of activity (Fig. 2b). This was followed, in order of decreasing importance, by the percentage of coarse basalt, the percentage of coarse basanite 2 and the percentage of fine basanite 2 (Fig. 1b). Similar to the percentage of fine basalt, having over 70% of coarse basalt led to a decrease in activity. In contrast, increasing the percentages of coarse basanite 2 led to a higher activity, while for fine basanite 2, activity increased with increasing percentages until 30%, after which adding more fine led to a stable predicted activity (Fig. 2b).

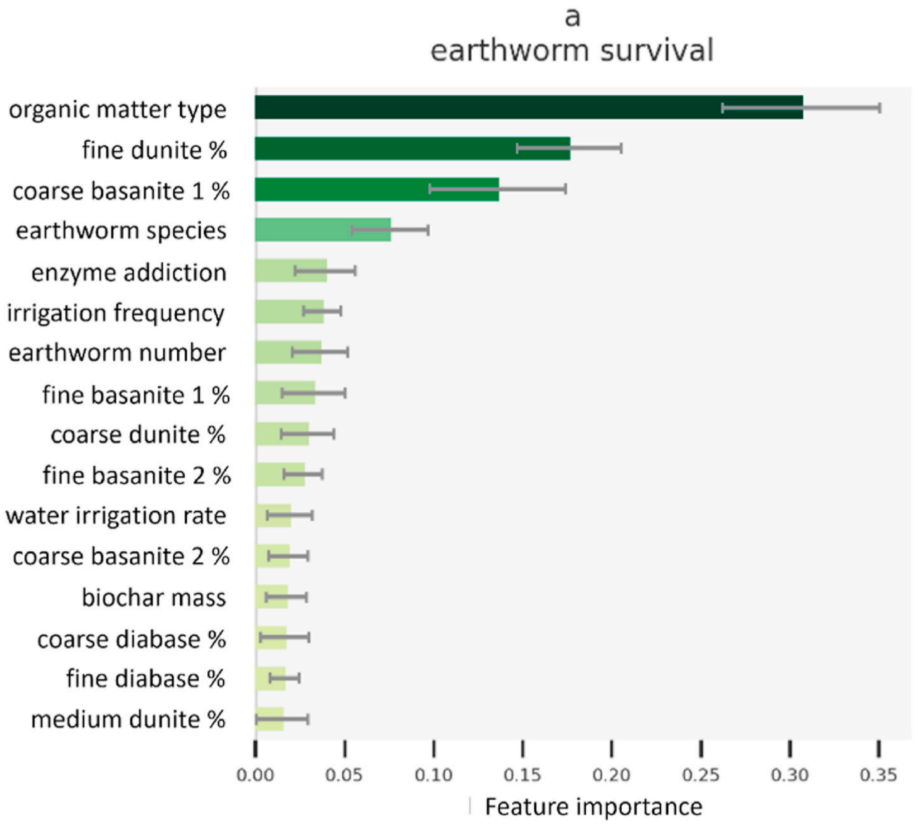
### 3.2. Earthworm effect on mineral weathering indicators

Earthworms did not significantly affect weathering indicators (p-value >0.05; Table 4). Only EC, Mg and P increased in the presence of earthworms. As in some treatments earthworms died during the experiment, potentially affecting weathering indicators, we analysed the effect of earthworms that survived and earthworms that died by the end of the experimental period. The presence of survived earthworms resulted in a significant decreased DIC, TIC, Ca, and Si (p-value <0.05; Table 6), exhibiting a medium effect size ($0.1 < r < 0.3$). The death of earthworms, instead, led to an increase in the amounts of almost all weathering indicators (p-value <0.05; Table 6), with a particular large effect size on DIC, TA, Ca and Mg ($r > 0.5$). Only ΔSIC, TIC, K and Si were not significantly influenced by earthworms that died (p-value >0.05).

## 4. Discussion

### 4.1. Optimal living conditions for earthworms in an artificial organo-mineral system

Earthworm survival and activity were mainly driven by features that directly influence the structure of the artificial mixture and thus also its drainage capacity, such as organic matter type, grain size and rock type (Fig. 1). Regarding the type of organic matter, earthworms generally prefer residues with low C:N ratio and in a more advanced decomposition stage (Hendriksen, 1990; Edwards and Arancon, 2022). Barley (1959) reported that *A. caliginosa* increased its body weight of between +71% and +111% when fed dung compared to plant litter, where the weight gain varied between +18% and −26%. The preference for straw that has a higher C:N ratio compared to co-digestate in the present study



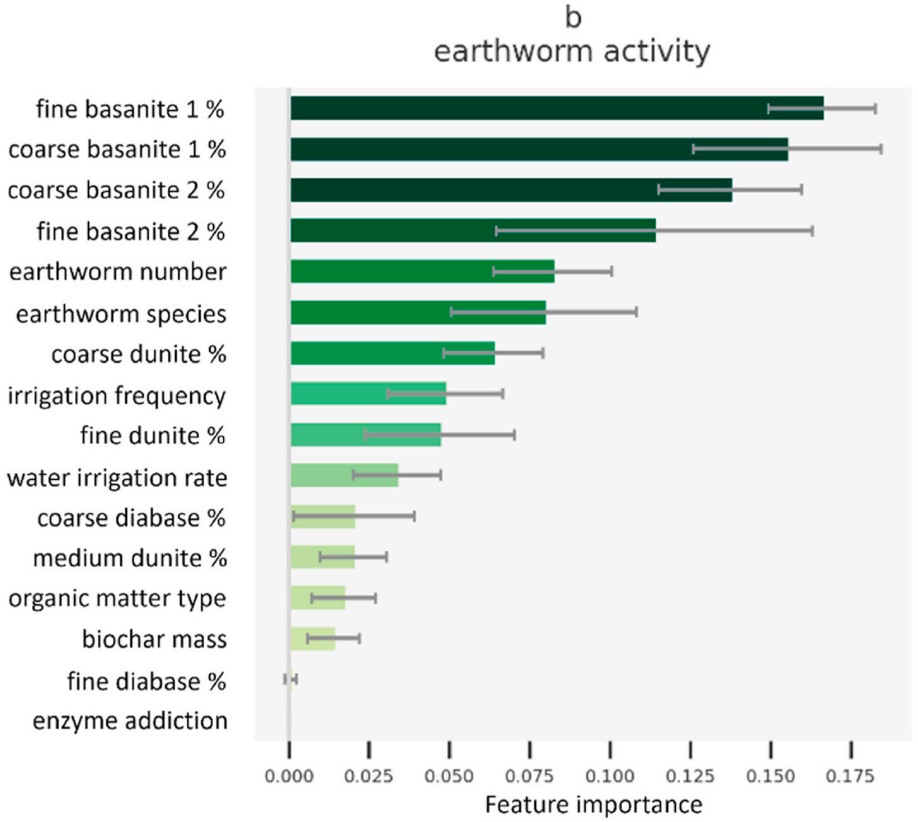


**Fig. 1.** Feature importance plots, showing the average importance of each feature as a predictor of earthworm survival (a) and earthworm activity (b) in the corresponding random forest models. Features are ranked from most (dark green) to least (light green) important. The coloured bars and the error bars represent the mean and the standard deviation of the feature importance in the 10 random forest models in the outer loop of the nested cross validation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

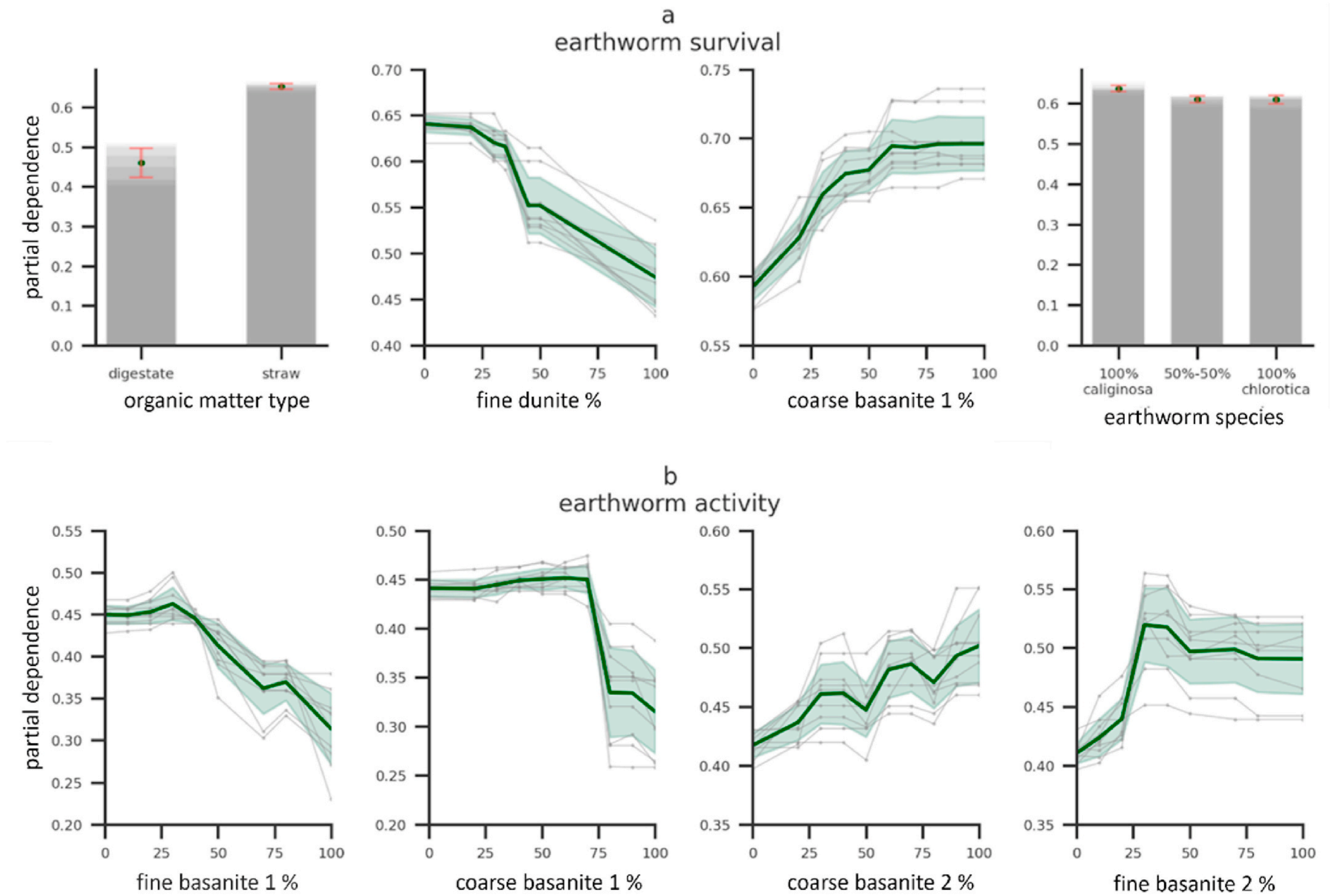


**Fig. 2.** Partial dependence plots (PDPs) indicating the dependence between earthworm survival (a) and earthworm activity (b), and the four features with the highest importance in the corresponding random forest models (see Fig. 1). For numerical variables, the grey lines indicate the partial dependences of the features in the 10 models in the outer loop of the nested cross validation. The thick green line indicates the mean of these 10 partial dependences and the light green shade the standard deviation. For categorical variables, the partial dependences of the features in the 10 models in the outer loop of the nested cross validation are indicated by the superimposed grey bars. Their mean values and standard deviations are indicated by the green dots and red error bars, respectively. PDPs are ordered based on decreasing feature importance from left to right. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

likely relates to improvements in the structure of the organo-mineral mixture rather than because of its nutritional value (Fig. 2a). Straw presented a more durable and voluminous structure, which could have prevented the compaction of the organo-mineral mixture compared to digestate that was more easily degradable and presented a less bulky structure.

Overall, decreasing percentages of fine grain sizes, and increasing percentages of coarse grain sizes, were beneficial for both survival and activity (Fig. 2). This contrasts with previous studies which found that earthworms generally prefer fine-textured soils in natural systems (Nordström and Rundgren, 1974; Edwards and Bohlen, 1996; Fraser et al., 1996; Edwards and Arancon, 2022). As the moisture content in our system was controlled and higher than in an average soil, coarser grain sizes likely facilitated drainage compared to fine grain sizes, favouring water flow and preventing the creation of anoxic conditions that are not favourable for earthworms (Zorn et al., 2008; Kiss et al., 2021a, 2021b; Edwards and Arancon, 2022). Additionally, the dependency of earthworm activity on the percentage of coarse minerals could be due to higher percentages likely facilitating earthworm movements through the system and therefore requiring less energy, especially for coarse basanite 2 (Fig. 2b). In the case of coarse basanite 1, activity decreased with percentages between 75% and 100%, probably because of the PSD of these rock grains which did not fall within the preferable size range of mineral particles on which these earthworm species generally feed (Abail et al., 2017).

Rock type also influenced earthworm activity, likely because of the different porosity of the rock grains and consequently different structure of the system. If the basanite 2 grains used in our experiment were more porous compared to the basanite 1, this might have favoured oxygen availability and water flow, and further a lower compaction of the organo-mineral mixture. Consequently, more suitable conditions for earthworms would have established when comparing fine basanite 2 to fine basanite 1.

Besides the structure of the organo-mineral mixture, earthworm species also impacted earthworm survival. The better survival of *A. caliginosa* in our system compared to *A. chlorotica* is in line with earlier studies showing that this species is more resistant and tolerates more extreme conditions (e.g. pH and flooding) than *A. chlorotica* (Zorn et al., 2008; Edwards and Arancon, 2022). Overall, combining factors that lead to a better structure of the organo-mineral mixture, such as specific organic residues and rock flours of coarser grain sizes and presumably higher porosity, can create the right conditions to ensure a higher earthworm survival. This results to be particularly important if we want to assess the effect of earthworms on weathering indicators according to their survival.

**Table 6**

Effect of earthworms on weathering indicators. Earthworm addition indicates all treatments where earthworms were introduced, survived earthworms indicate the treatments where the survival rate was higher than 75 %, while dead earthworms indicate the treatments where the survival rate was lower than 25 %. Grey colouring indicates no significant effect (p-value >0.05), green a positive effect and yellow a negative effect (p-value <0.05). The effect size (r) was calculated for indicators with a p-value <0.05. DIC = dissolved inorganic carbon; ΔSIC = change in solid inorganic carbon; TIC = total inorganic carbon; TA = total alkalinity; EC = electrical conductivity; $[H^+]$ = hydrogen ion concentration; DOC = dissolved organic carbon; Ca = calcium; Mg = magnesium; K = potassium; P = phosphorus; Fe = iron; Si = silicon.

| Weathering indicator | Earthworm addition | | Survived earthworms | | Dead earthworms | |
|---|---|---|---|---|---|---|
| | *p-value* | *r* | *p-value* | *r* | *p-value* | *r* |
| DIC | 0.190 | - | 0.036 | 0.358 | 0.001 | 0.689 |
| ΔSIC | 0.182 | - | 0.086 | - | 0.100 | - |
| TIC | 0.674 | - | 0.044 | 0.342 | 0.979 | - |
| TA | 0.183 | - | 0.153 | - | 0.001 | 0.696 |
| EC | 0.01 | 0.307 | 0.153 | - | 0.028 | 0.467 |
| $[H^+]$ | 0.957 | - | 0.215 | - | 0.046 | 0.426 |
| DOC | 0.373 | - | 0.460 | - | 0.008 | 0.551 |
| Ca | 0.393 | - | 0.005 | 0.458 | 8.059e-05 | 0.765 |
| Mg | 0.047 | 0.237 | 0.162 | - | 0.0002 | 0.737 |
| K | 0.868 | - | 0.428 | - | 0.524 | - |
| P | 0.001 | 0.392 | 0.055 | - | 0.010 | 0.561 |
| Fe | 0.273 | - | 0.635 | - | 0.043 | 0.433 |
| Si | 0.327 | - | 0.044 | 0.335 | 0.443 | - |

### 4.2. *Survived earthworms versus dead earthworms matter for weathering indicators*

Earthworms that survived did not have a significant positive effect on any weathering indicator, and a medium negative effect on DIC, TIC, Ca, and Si ($0.3 < r < 0.5$; Table 6). Part of our findings are in line with Vienne et al. (2024), who carried out a mesocosm experiment with earthworms, soil, and basalt. After 137 days of experiment, these authors did not find a significant effect of earthworms on SIC and a decrease in DIC as we did, but in contrast to our work they detected an increase in Ca in the leachate. They also found an increase in soil exchangeable Ca, K and Mg and cation exchange capacity (CEC) of 35.7, 38.7, 72.3 and 29.6%, respectively, in basalt treatments where earthworms were added. These findings could highlight the role of earthworms in stimulating weathering and/or in increasing organic matter stabilization (Vienne et al., 2024). The duration of our experiment might have been too short to detect changes in SIC, while earthworms could have still enhanced weathering (Fig. 3). Additionally, the decrease in leached DIC, Ca and Si in our results might have been due to a higher retention of these elements because of an increase in the CEC of the organo-mineral mixture, in line with the findings of Vienne et al. (2024). A higher CEC could have been a consequence of the formation of new clay minerals induced by earthworms (Carpenter et al., 2007; Hodson et al., 2014). This could have further enhanced formation and retention of negatively charged organic matter (OM), which could have occurred at the relatively high pH in our system (average pH = 8.36) (Fig. 3a; Lu et al., 2015; Sarkar et al., 2018; Solly et al., 2020; Bi et al., 2023). Besides an increase in OM protection (Baldock and Skjemstad, 2000), the binding of OM could have led to a consumption of alkalinity, further decreasing DIC (Fig. 3; Vienne et al., 2024). Therefore, changes in DIC might be indirectly driven by changes in both SIC and OM.

Unlike earthworms that survived, earthworms that died by the end of the experiment significantly increased almost all leachate-derived weathering indicators in our experiment, with a particularly stronger effect on DIC, TA, Ca, Mg and P ($r > 0.5$), but did not increase SIC (Table 6). In our artificial system, dead earthworm bodies likely released decomposition products, resulting in the increase in weathering indicators through direct and indirect pathways (Fig. 3b). Decaying earthworm tissue releases nutrients and organic carbon already within the first three days of decomposition (Whalen et al., 1999; Sun and Ge, 2021; Trap et al., 2021). As a result, earthworm bodies' decay can enhance the activity and specific diversity of microbial communities coming from the surrounding environment as well as from earthworm guts (Kos et al., 2017; Sun and Ge, 2021; Lin et al., 2022), including microbes known for solubilizing minerals that dwell in earthworm intestines (Hu et al., 2018; Liu et al., 2018). An increased microbial activity might have resulted in higher organic matter decomposition, which would have led to a higher $CO_2$ release (Ajwa and Tabatabai, 1994; Condron et al., 2010). The release of $CO_2$ in the presence of water leads to the formation of carbonic acid and further enhances carbonation weathering in the system (Amann et al., 2022). This leads to the release of additional cations, as Ca and Mg, and, under the pH values observed in the leachates of the columns (ranging between 7.65 and 9.06), results in the formation of bicarbonate and carbonate ions, further increasing DIC and TA (Middelburg et al., 2020). In addition, the enhanced activity of microorganisms might have led to the release of organic acids, which are known, besides increasing organic matter decomposition, to stimulate weathering, further explaining higher $[H^+]$ (Ribeiro et al., 2020). Finally, earthworm bodies have likely released $CO_2$ during the decomposition of animal tissues (Keenan et al., 2018). The $CO_2$ released could have also contributed to enhance weathering through carbonic acid formation as explained above.

### 4.3. *Do earthworms have the potential to accelerate ESW in bioreactors?*

The choice of methodology is crucial when aiming at understanding the effect of earthworms on ESW, especially for its potential use as a CDR technology in bioreactors to increase carbon sequestration. Studies

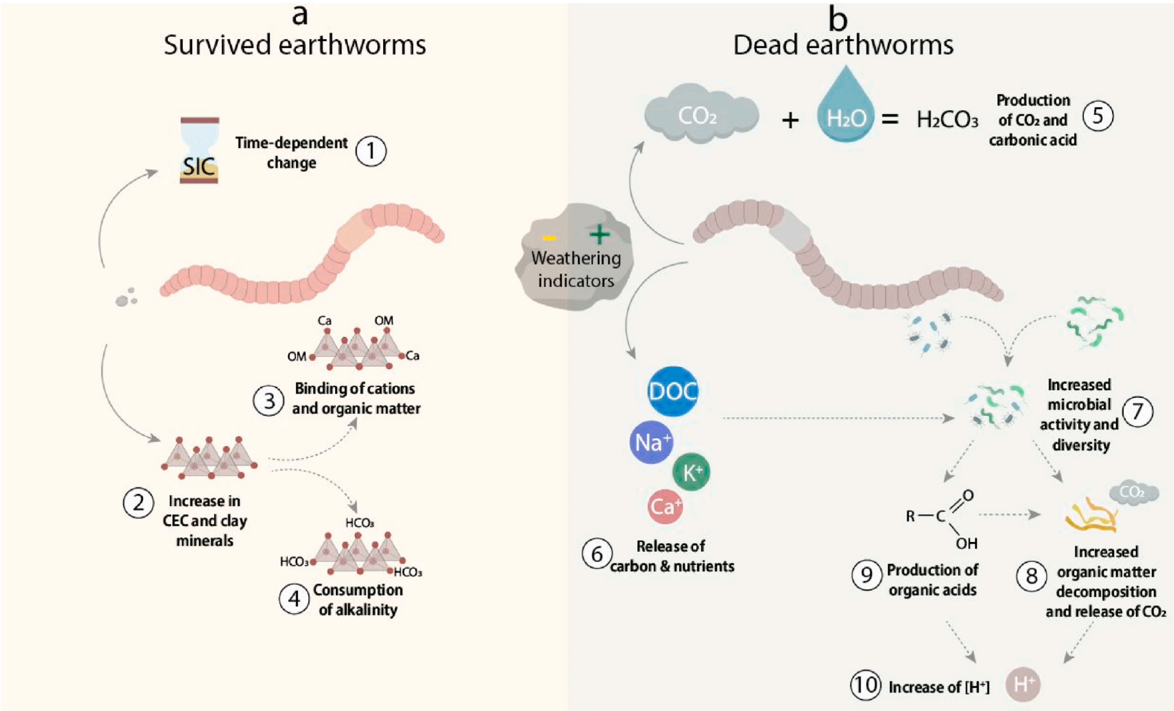


**Fig. 3.** Potential pathways through which survived (a) and dead (b) earthworms affect weathering indicators. Solid arrows indicate direct effects, while dashed arrows indicate indirect effects of earthworms on weathering indicators. The effect of survived earthworms on SIC is time dependent (1) and may decrease weathering indicators due to secondary processes such as the formation of clay minerals (2). This might influence the cation exchange capacity of the organo-mineral mixture, retaining major cations and organic matter (3), besides consuming alkalinity (4). Dead earthworms enhance weathering indicators due to: release of carbon dioxide ($CO_2$) (5) and organic carbon and nutrients (6) during decomposition of earthworm bodies; increased microbial activity and diversity (7); consequent increased organic matter decomposition and release of $CO_2$ (8); increased organic acids production (9), which, together with $CO_2$ production leads to an increase in hydrogen ($H^+$) ions (10).

demonstrating a positive effect of earthworms on mineral weathering have primarily focused on changes in mineralogy (Carpenter et al., 2007) or in grain shape (Suzuki et al., 2003). Changes in mineralogy assessed through XRD or imaging techniques as scanning-electron microscopy (SEM) could be indicative of weathering, but they do not provide insights on the fate of weathering products and on the potentially sequestered carbon. Relying solely on mineralogical changes might lead to an overestimation of earthworms' potential to enhance inorganic carbon sequestration through silicate weathering. To accurately determine the potential of earthworms on weathering as a CDR technology, it is necessary to quantify CDR directly via measuring IC, or indirectly using other indicators for carbon sequestration such as pH, EC, and element release. The few studies that monitored weathering products to evaluate the effect of earthworms on weathering showed mixed results, revealing either a negative or neutral effect (Liu et al., 2011; Zhu et al., 2013; Vienne et al., 2024), even if indications of increased weathering were found (Vienne et al., 2024). The difference between studies highlights that, while earthworms might increase weathering, this does not necessarily translate into greater inorganic carbon sequestration. Other overlooked earthworm-driven weathering processes, such as effects on organic matter stabilization and consequent protection of organic carbon through weathering, might dominate over inorganic carbon sequestration.

## 5. Conclusions

The importance of our study lies in being the first exploratory study aiming at enhancing mineral weathering through the activity of earthworms while controlling a series of other abiotic conditions known for stimulating weathering in controlled small-scale reactors. This study aimed at determining the optimal living conditions for earthworms in an artificial organo-mineral system simulating a bio-reactor while concurrently evaluating the effect of earthworms on weathering indicators. The results showed that earthworm survival and activity mainly depended on a series of features that directly influenced the structure and drainage capacity of the organo-mineral system in simulated bioreactors. The type of organic matter, rock type and grain size were the main features governing earthworm survival and activity. Specifically, straw, basanite 2 rock and coarser grain sizes led to better conditions for earthworms in this artificial system by improving the structure of the organo-mineral mixture, further facilitating earthworm movements through the system and preventing the development of anoxic conditions. In general, we did not observe a clear effect of earthworms on weathering indicators. When considering the survival rate of earthworms, we found that earthworms that survived had no or even a negative effect on weathering indicators, while earthworms that died during the experimental period enhanced these parameters. We propose some conceptual pathways through which decaying earthworm bodies could enhance weathering indicators amounts: (1) production of $CO_2$ and release of carbon and nutrients during decomposition; (2) enhanced activity of microorganisms coming from earthworm bodies themselves and from their surroundings; (3) production of organic acids by microbes; (4) increased organic matter decomposition. Furthermore, depending on the research goal, we highlight the need to quantify the role of earthworms, and in general of soil biota, on ESW for CDR through a series of standardised common weathering indicators aimed at quantifying carbon removal, such as DIC, SIC and TA. Our study highlights that even if changes in mineralogy are indicative of mineral weathering, earthworms may not be suitable to enhance silicate weathering in reactors for carbon sequestration. To accurately assess the true effect of earthworms on carbon sequestration due to enhanced silicate weathering in bioreactors and other experimental settings, it is essential to directly quantify inorganic carbon or measure weathering indicators, such as total alkalinity.

## CRediT authorship contribution statement

**Tullia Calogiuri:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Iris Janssens:** Writing – review & editing, Visualization, Software, Methodology. **Alix Vidal:** Writing – review & editing, Methodology, Conceptualization. **Jan Willem Van Groenigen:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Tim Verdonck:** Writing – review & editing, Methodology, Funding acquisition. **Thomas Corbett:** Writing – review & editing, Methodology, Investigation, Data curation. **Jens Hartmann:** Writing – review & editing, Methodology, Funding acquisition. **Anna Neubeck:** Writing – review & editing, Methodology, Funding acquisition. **Harun Niron:** Writing – review & editing, Methodology, Investigation, Data curation. **Reinaldy P. Poetra:** Writing – review & editing, Methodology, Investigation, Data curation. **Lukas Rieder:** Writing – review & editing, Methodology, Investigation, Data curation. **Thomas Servotte:** Writing – review & editing, Methodology. **Abhijeet Singh:** Writing – review & editing, Methodology, Investigation, Data curation. **Michiel Van Tendeloo:** Writing – review & editing, Methodology. **Siegfried E. Vlaeminck:** Writing – review & editing, Methodology, Funding acquisition. **Sara Vicca:** Writing – review & editing, Methodology, Funding acquisition. **Mathilde Hagens:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization.

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## Declaration of competing interest

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apgeochem.2024.106271.

## Data availability

The datasets generated and analysed during the current study are available in Calogiuri et al. (2024) in the Pangaea repository at https://doi.pangaea.de/10.1594/PANGAEA.968993. The dataset "Factors governing earthworm survival and activity in an artificial organo-mineral system" (doi: 10.1594/PANGAEA.968961), was used to determine the best living conditions for earthworms in the artificial organo-mineral system. The dataset "Weathering indicators according to earthworm presence and survival in an artificial organo-mineral system" was used to determine the effect of earthworms on weathering indicators.

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