Pedobiologia 54S (2011) S119–S129

Contents lists available at SciVerse ScienceDirect

Pedobiologia - International Journal of Soil Biology

journal homepage: www.elsevier.de/pedobi

9th International Symposium on Earthworm Ecology

Production and dissolution rates of earthworm-secreted calcium carbonate

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ARTICLE INFO

Article history:
Received 14 October 2010
Received in revised form 8 September 2011
Accepted 10 September 2011

Keywords:
Earthworm
Calcium carbonate
Production
Dissolution
Lumbricus terrestris
Carbon
Fluxes
Calcium
Biogeochemical cycles

ABSTRACT

Earthworms secrete granules of calcium carbonate. These are potentially important in soil biogeochemical cycles and are routinely recorded in archaeological studies of Quaternary soils. Production rates of calcium carbonate granules by the earthworm *Lumbricus terrestris* L. were determined over 27 days in a range of soils with differing chemical properties (pH, organic matter content, water holding capacity, bulk composition, cation exchange capacity and exchangeable cations). Production rate varied between soils, lay in the range 0–0.043 mmol_{CaCO_3} (0–4.3 mg) earthworm^{-1} d^{-1} with an average rate of 8×10^{-3} mmol_{CaCO_3} (0.8 mg) earthworm^{-1} d^{-1} and was significantly correlated ($r = 0.68$, $P \leq 0.01$) with soil pH. In a second experiment lasting 315 days earthworms repeatedly (over periods of 39–57 days) produced comparable masses of granules. Converting individual earthworm granule production rates into fluxes expressed on a per hectare of land per year basis depends heavily on estimates of earthworm numbers. Using values of 10–20 *L. terrestris* m^{-2} suggests a rate of 18– 3139 mol_{CaCO_3} ha^{-1} yr^{-1}. Data obtained from flow-through dissolution experiments suggest that at near neutral pH, granule geometric surface area-normalised dissolution rates are similar to those for other biogenic and inorganic calcites. Fits of the data to the dissolution relationship $r = k(1-\Omega)^n$ where r = dissolution rate, k = a rate constant, Ω = relative saturation and n = the reaction order gave values of $k = 1.72 \times 10^{-10}$ mol cm^{-2} s^{-1} and $n = 1.8$ for the geometric surface area-normalised rates and $k = 3.51 \times 10^{-13}$ mol cm^{-2} s^{-1} and $n = 1.8$ for the BET surface area-normalised rates. In 196 day leaching column experiments trends in granule dissolution rate referenced to soil chemistry corresponded to predictions made by the SLIM model for dissolution of limestone in soil. If soil solution approaches saturation with respect to calcium carbonate, granule dissolution will slow or even stop and granules be preserved indefinitely. Granules have the potential to be a small but significant component of the biogeochemical cycling of C and Ca in soil.

Introduction

Earthworms have been called ecosystem engineers and play a key role in delivering many ecosystem services through their burrowing, soil mixing and role in organic matter decomposition (Lavelle et al. 2006). Many species of earthworm are also known to secrete granules of calcium carbonate which may be up to 2 mm in diameter (Canti and Piearce 2003). The calcium carbonate is present predominantly as calcite but also as aragonite, vaterite and amorphous calcium carbonate (Gago-Duport et al. 2008; Lee et al. 2008). The function that calcium carbonate granule secretion serves for the earthworm is unknown with suggestions linking it to excretion of excess Ca, neutralisation of gut pH and regulation of CO_2 (Robertson 1936; Piearce 1972a; Darwin 1881; Briones et al. 2008).

Previous studies have shown that earthworm granules are commonplace in soils (Ponomareva 1948; Wiecek and Messenger 1972; Bal 1977; Canti 1998). On the basis of field measurements Wiecek and Messenger (1972) estimated that excreted calcium carbonate could contribute up to 11 mol_{CaCO_3} ha^{-1} yr^{-1} to forest soils. Canti and Piearce (2003) determined that granule production rates were greatest for the earthworms *Lumbricus terrestris* L. and *Lumbricus rubellus* Hoffmeister out of 8 species studied. Canti (2007) estimated production rates of "more than" 0.02 mmol_{CaCO_3} (i.e. 2 mg) d^{-1} per *L. terrestris*.

Balancing production of granules is their dissolution. Granules have been recorded in Quaternary soils and archaeological materials (Canti 1998) but presumably they have a finite life in soils. Whilst there is much data in the literature on the dissolution of calcite (e.g. Sjöberg 1976; Morse 1978; Keir 1980; Walter and Morse

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doi:10.1016/j.pedobi.2011.09.003

1984; Walter 1985; Jordan and Rammensee 1998; Duckworth and Martin 2004; Cubillas et al. 2005), little consideration has been given to rates of calcite dissolution in soils (Elphick 1955; Warfvinge and Sverdrup 1989) nor is it known whether granules dissolve at the same rate as other calcium carbonates.

The purpose of this study was therefore threefold. Firstly, to determine the influence of soil chemistry on calcium carbonate granule production rates by the earthworm *L. terrestris*, secondly to carry out a preliminary investigation into granule dissolution rates and thirdly to collate this data to determine whether granules are likely to be important in the biogeochemical cycling of C and Ca.

Materials and methods

Production experiments

Eleven different topsoils (0–30 cm) were collected from the area local to Reading (see Table 1 for precise locations), air-dried, sieved to <250 μm and fully characterised prior to experiments to ensure that they had contrasting chemical properties. The soils have a range of pH, organic matter content, cation exchange capacities and exchangeable cations (Table 1). X-ray diffraction indicated that the dominant mineralogy of all the <250 μm soils was quartz (>90 wt%) apart from Coombe Complex which contained 42 wt% calcite and 12 wt% dolomite. All soils contained minor amounts of alkali feldspar (<5 wt%) and kaolinite, haematite and muscovite (<5 wt%). Clitellate *L. terrestris* (mass = 5.01 ± 0.14, mean ± standard error, n = 62) were purchased from Worms Direct (Drylands, Ulting, Nr Maldon, Essex, CM9 6QS, UK) and kept in a moist mixture of 33% by dry volume peat and 67% by dry volume Kettering loam (Boughton Loam and Turf Management, Kettering, Northamptonshire, NN16 8UN, UK) for one week prior to experiments.

In the main production experiment, in each experimental unit one (weighed) *L. terrestris* was added to moist soil (300 g air dry soil plus sufficient deionised water to raise the soil to 65% of its water holding capacity). Three grams of finely ground (≤250 μm) moist horse manure were added to each earthworm container at the start of the experiment as food. Moisture content was kept constant throughout the experiment by addition of deionised water if treatments lost weight. Treatments were kept at 18 °C in a temperature controlled laboratory. After 27 days earthworms were removed and weighed prior to release. The soil was wet-sieved to 500 μm allowing recovery of granules >500 μm in size from the soil. These were air dried and weighed. Six replicates were used for each soil. In trial experiments 500–250 μm granules were also retained and weighed. Whilst granules were recovered in this fraction they represented a negligible fraction of the total mass of granules recovered. Therefore, given the increased difficulty and time involved in sieving to this finer fraction, 500 μm was used as the cut off for granule recovery in the main production experiments.

To further investigate production rates a second experiment was established in which eight *L. terrestris* earthworms were each separately and repeatedly kept in Hamble soil (Table 1) following the same methodology as outlined above for periods of 39–57 days in order to determine whether granule production rates per earthworm were constant. The Hamble soil was chosen because earthworms produced large numbers of granules in it, there was a large amount of this soil available and it proved the easiest of our 11 soils to sieve to <250 μm. After each exposure period granules were extracted from the soil, earthworms were weighed and then transferred to fresh Hamble soil. This process was repeated a total of seven times over 315 days. Three grams of moist, finely ground horse manure were added to each container every 14 days as a food source.

Dissolution experiments

For the dissolution experiments granules and Iceland spar calcite (as a control) were used (Fig. 1). Granules were produced by *L. terrestris* earthworms kept in Hamble soil and then collected following the same protocol as in the production experiments except that the soil was kept at a moisture content of 85% of the water holding capacity. The Iceland spar used in the experiments was obtained from our departmental collection. It was ground, sieved to produce a 150–212 μm size fraction and cleaned in an ultrasonic water bath. Prior to dissolution the granules and Iceland spar were confirmed as calcite using X-ray diffraction. Previous work (Lee et al. 2008) had determined the chemical composition of the granules produced in the Hamble soil. Whilst the granules contained trace amounts of elements other than Ca (e.g. 352 mg kg^{-1} Mg, 174 mg kg^{-1} Mn) these concentrations are unlikely to impact on dissolution rates making Iceland Spar an appropriate inorganic control. BET (Brunauer, Emmet and Teller) surface areas were determined using a Gemini III 2375 Surface area analyser to perform nitrogen absorption and apply the BET (Brunauer, Emmet and Teller) isotherm (Brunauer et al. 1938); geometric surface areas were calculated assuming the granules and Iceland spar were smooth spheres and cubes of diameter 1125 μm and length 181 μm respectively (Table 2). Accuracy of the BET surface area determinations was confirmed by analysis of the Micromeritics kaolinite standard 004-16819-00 for which results were within the stated range of 16.3 ± 0.8 $m^2\,g^{-1}$ for multipoint BET determinations.

Flow through reactors: Air-dried granules or Iceland spar calcite were dissolved in flow-through reactors at 20 °C using $NaHCO_3$–NaCl–HCl solution to buffer atmospheric CO_2 and control pH. The alkalinity of randomly selected reacted solutions was determined by titration against HCl and confirmed that this was successful; alkalinity was as expected for the concentration of $NaHCO_3$ in solution and constant through experimental runs. Experimental conditions are detailed in Table 3 for each experimental run. Experiments were conducted in closed 150 ml polypropylene vessels which contained a floating Teflon-covered magnetic stirrer and had a minimal air-filled headspace of <10 ml. Reactive solution was stored in an air-free reservoir and pumped into the reactors using a Watson–Marlow peristaltic pump at a rate of 0.5–2 ml per minute. pH of reacted fluids was measured in-line every 5 min before collecting solutions using a fraction collector. The pH meter was calibrated using commercial buffers (pH 4.00 and 7.00) and the calibration was checked at the beginning and end of each experimental run. Ca concentration of the reacted fluid was measured using a Perkin Elmer Optima 3000 inductively coupled plasma-optical emission spectrometer (ICP-OES). Analytical precision based on repeat analyses was ±5% and detection limit was 6 μg l^{-1}. Accuracy was assessed by analysis of an in-house matrix matched standard and was ±10%. A steady state dissolution rate was assumed to have been achieved when outlet solution Ca concentration was constant within error for at least two residence times in the reactors. Steady state was achieved sufficiently rapidly in the Iceland spar experiments that flow rate was changed and the experiment continued until a second steady state was established using the same reaction fluid and calcite sample throughout.

Saturation of solutions with respect to calcite and other phases under steady state conditions was calculated using the PHREEQC for Windows, Version 2.15.07 computer code (Parkhurst and Appelo 1999) with the PHREEQC database. Dissolution rates were calculated using:

$$r = \frac{f \times m_{Ca}}{M \times S} \tag{1}$$

where r is the dissolution rate (mol$CaCO_3$ $cm^{-2}\,s^{-1}$), f is the flow rate through the reactor (ml s^{-1}), m_{Ca} is the Ca concentration at