# Soil pH governs production rate of calcium carbonate secreted by the earthworm *Lumbricus terrestris*

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**ABSTRACT**

*Lumbricus terrestris* earthworms exposed to 11 soils of contrasting properties produced, on average, 0.8 $\pm$ 0.1 $mg_{calcite}$ $earthworm^{-1}$ $day^{-1}$ in the form of granules up to 2 mm in diameter Production rate increased with soil pH ($r^2 = 0.68$, $p \leq 0.01$). Earthworms could be a significant source of calcite in soils.

## 1. INTRODUCTION

Earthworms secrete granules of calcium carbonate, predominantly calcite but also aragonite, vaterite and amorphous calcium carbonate (Gago-Duport et al., 2008; Lee et al. 2008). The granules are produced in the calciferous glands of the earthworm. In the case of *L. terrestris* these occur in segments 10, 11 and 12 as three pairs of swellings off the oesophagus. Micron-scale “spherites” of amorphous calcium carbonate are secreted in the rear two pairs of oesophagal glands and move forwards into a pair of oesophagal pouches, by which time they have largely crystallised to calcite and have combined to form granules. The granules are secreted from the oesophagal pouches through a sphincter into the oesophagus. The function that these granules serve for the earthworm is unknown (Darwin, 1881; Robertson, 1936; Piearce, 1972; Briones et al., 2008) but 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 $mol_{CaCO3}$ $ha^{-1}$ $yr^{-1}$ to forest soils. Canti and Piearce (2003) determined that granule production rates were greatest for the earthworms *Lumbricus terrestris* and *L. rubellus.* Canti (2007) estimated production rates of 2.2 $mg_{calcite}$ $day^{-1}$ $earthworm^{-1}$ for *L. terrestris.* The aim of the study reported here was to determine how granule production rate of *L. terrestris* varied with soil properties.

## 2. METHODS

Eleven soils were collected, air-dried, sieved to < 250 μm and characterised (Table 1). In all production experiments one (weighed) clitellate *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). Moisture content was kept constant throughout the experiment by addition of deionised water if treatments lost weight. Treatments were kept at 18 °C under ambient light conditions. At the end of each experiment the earthworm was removed and weighed prior to release. The soil was wet-sieved to 500 µm to recover freshly produced granules from the soil. These were air dried and weighed.

In our first experiment earthworms were exposed to the 11 different soils for 27 days with six replicates per treatment. Three grammes of horse manure were added to each earthworm container at the start of the experiment. In a second experiment eight earthworms were each separately and repeatedly exposed to Hamble soil for periods of 39 - 57 days. 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 grammes of horse manure were added to each container every 14 days. Data were checked for normality and equality of variance. In the first experiment treatments were compared using Kruskall-Wallis one-way analysis of variance on ranks and Dunn's method for pairwise comparisons. In the second experiment reported correlations are Pearson correlations. All statistical analysis used SigmaStat for Windows 3.01 produced by SPSS inc. In the text values are expressed as mean $\pm$ standard error.

## 3. RESULTS

At the end of the first experiment one earthworm had escaped or died from the Coombe Complex, Kettering Loam, Neville, Park Gate and St Albans Wood soils and two from the Hamble replicates. Granule production varied both within and between soils ($p \leq 0.01$) (Fig. 1). Within individual soils there was on average a factor of 7.7 $\pm$ 2.2 (n = 10, St Albans Wood data discounted due to an absence of granules) times difference in masses of granules produced by individual earthworms in the six replicate containers. Across all soils, granule production rate varied between 0 in the pH 4.3 St Albans Wood soil (the next lowest production rate was 0.05 $mg_{CaCO3}$ $earthworm^{-1}$ $day^{-1}$ in one of the pH 6.1 Wilderness soil replicates) and 4.3 $mg_{calcite}$ $earthworm^{-1}$ $day^{-1}$ (in one of the Coombe Complex soil replicates, pH 7.8). Average production rate for all soils was 0.8 $\pm$ 0.1 $mg_{CaCO3}$ $earthworm^{-1}$ $day^{-1}$ (n = 57). Of the soil properties listed in Table 1, granule production rate was strongly correlated with pH ($r^2$ = 0.68, $p \leq 0.01$) but none of the other measured soil properties.

In the second experiment, over the 315 days two deaths occurred (between adjacent sampling dates of 81 and 123 days) and the earthworms lost weight from an initial 5.3 $\pm$ 0.5 g (n = 8,) to 2.4 $\pm$ 0.3 g (n = 6) at the end of the experiment. There was a significant correlation between production rate and earthworm mass (r = 0.62, $p \leq 0.01$). Three individual earthworms (earthworms 1, 3 and 5), which showed a significant correlation between granule production and earthworm mass (r = 0.65 to 0.92, $p \leq 0.01$), also showed a significant negative correlation between granule production and time (r = -0.78 to -0.93, $p \leq 0.01$) (Fig. 2). On average granule production rate, expressed on a $mg_{calcite}$ $earthworm^{-1}$ $day^{-1}$ basis, varied between earthworms by a factor of 3.4 $\pm$ 0.6 (n = 6); normalising to earthworm mass (i.e. $mg_{calcite}$ $g^{-1}{}_{earthworm}$ $day^{-1}$) did not significantly reduce this level of variation (3.3 $\pm$ 0.5).

## 4. DISCUSSION

Granule production rate shows significant variation between earthworms and between soils. The variation, at least in part, appears to reflect naturally occurring biological variation between individual earthworms with larger earthworms producing more granules. However, there are significant differences in production rates between soils, which indicates that some of the variation is due to differences in soil properties. Whilst the correlation between production rate and pH potentially reflects dissolution of the granules in the soil, granule dissolution rates are not sufficiently rapid for this to be the case (Lambkin et al., this issue). The hypothesis that the low soil pH lowers the saturation state of calcium carbonate in the earthworm calciferous glands thereby limiting granule production is attractive. However, the oesophagal glands where the granules are initially precipitated as spherites have no direct contact with the soil and it seems unlikely that the fluids from which the spherites precipitate would reflect the soil pH as they will have a homeostatically regulated pH. Set against this we have observed inclusions of quartz and feldspar in granules (Lee et al. 2008) so there must be some “leakage” of soil material into the calciferous glands, presumably this occurs as the sphincter opens and a granule is expelled from the glands into the oesophagus. Typically in low pH soils there is less Ca (either total or available) and this could limit granule production.

However, in these experiments there was no discernable relationship between granule production and either total Ca levels in the soil or Ca present on exchange sites. Thus the cause of the relationship between soil pH and granule production remains unresolved.

An average value for earthworm density is 270 earthworms $m^{-2}$, which covers a wide range of species in temperature climates (Edwards and Bohlen, 1996); though not all earthworm species produce granules. Using this value, granule production rates reported here of 0.05 to 0.8 to 4.3 $mgCaCO_3$ $earthworm^{-1}$ $day^{-1}$ (minimum, average and maximum values) correspond to production rates of 493, 7 884 and 42 377 $molCaCO_3$ $ha^{-1}$ $yr^{-1}$. A more conservative estimate of 10 to 20 *L. terrestris* per $m^2$ (Briones et al. 2008) yields production rates of 18 to 3139 $molCaCO_3$ $ha^{-1}$ $yr^{-1}$ which are more similar to the estimates of Wiecek and Messenger (1972) for forest soils. The precise amount of calcite produced annually by earthworms clearly depends on estimates of earthworm numbers as well as soil properties; however, the production rates of individual earthworms suggests to us that earthworm calcite production is a potentially important source of calcite in soils.

## 5. CONCLUSIONS

Biological variation has a significant impact on the mass of calcium carbonate secreted by earthworms in the form of granules; however, soil properties do play a role as well. The mass of granules produced by earthworms is significant and they are worthy of further study to improve our understanding of their significance in the terrestrial C cycle.


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Table 1. Mean chemical characteristics of the < 250 µm soils used in experiments expressed in terms of oven dry soil (n = 3 ± standard error except for elemental composition where n = 1)

| Name[1] | Sample site | pH | LOI[3] | WHC[4] | CEC[5] | Elemental composition[6] / wt % | | | | | | | | Exchangeable ions[7] / mg kg$^{-1}$ | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | $Al_2O_3$ | CaO | $Fe_2O_3$ | $K_2O$ | MgO | $Na_2O$ | $P_2O_5$ | $SiO_2$ | Al | Ca | Fe | K | Mg | Na | P |
| Coombe Complex | SU625733 | 7.8 ± 0.0 | 10.2 ± 0.1 | 54.7 ± 1.8 | 17.2 ± 0.4 | 6 | 39 | 4 | 1 | 3 | 0 | 0 | 45 | 1.1± 0.1 | 1933.6 ± 133.6 | 0 | 88,2 ± 5.9 | 59.3 ± 2.4 | 12.0 ± 2.8 | 36.6 ± 0.2 |
| Frilsham | SU596726 | 7.0 ± 0.0 | 7.7 ± 0.1 | 48.7 ± 1.9 | 16.6 ± 0.2 | 8 | 1 | 3 | 2 | 0 | 0 | 0 | 84 | 1.6 ± 0.0 | 3401.2 ± 9.2 | 0 | 142.5 ± 0.3 | 78.1 ± 0.2 | 9.3 ± 0.8 | 50.2 ± 0.7 |
| Hamble | SU618702 | 7.9 ± 0.0 | 4.2 ± 0.0 | 40.8 ± 0.8 | 10.7 ± 0.1 | 8 | 1 | 3 | 2 | 1 | 0 | 0 | 84 | 1.3 ± 0.0 | 2597.4 ± 15.3 | 0 | 166.2 ± 1.1 | 35.0 ± 0.2 | 5.9 ± 1.1 | 28.3 ± 0.1 |
| Kettering | N/A[2] | 7.4 ± 0.0 | 8.8 ± 0.1 | 53.8 ± 1.0 | 24.5 ± 0.5 | 14 | 2 | 8 | 2 | 1 | 0 | 0 | 71 | 2.1 ± 0.1 | 4948 ± 14 | 0.2 ± 0.0 | 196.9 ± 1.6 | 125.6 ± 0.4 | 20.1 ± 0.8 | 10.7 ± 0.4 |
| Neville | SU765754 | 5.4 ± 0.0 | 11.2 ± 0.1 | 52.0 ± 0.1 | 12.2 ± 0.7 | 6 | 1 | 4 | 2 | 0 | 0 | 1 | 84 | 1.3 ± 0.0 | 2533.7 ± 5.4 | 0.2 ± 0.0 | 473.2 ± 3.4 | 262.4 ± 0.4 | 13.3 ± 1.6 | 102.9 ± 1.0 |
| Parkgate | SU601684 | 5.6 ± 0.0 | 8.5 ± 0.1 | 61.0 ± 2.8 | 15.4 ± 0.3 | 10 | 0 | 4 | 2 | 1 | 0 | 0 | 81 | 1.3 ± 0.0 | 2490.9 ± 3.6 | 0.3 ± 0.0 | 478.0 ± 3.5 | 303.8 ± 0.4 | 19.2 ± 0.5 | 79.0 ± 0.1 |
| Soil Science | SU731718 | 6.5 ± 0.0 | 19.2 ± 0.2 | 71.2 ± 1.0 | 37.4 ± 0.1 | 9 | 2 | 6 | 2 | 1 | 0 | 0 | 79 | 89.9 ± 0.8 | 5515.8 ± 17.2 | 88.8 ± 1.7 | 455.2 ± 11.7 | 312.7 ± 1.5 | 307.8 ± 1.9 | 20.4 ± 0.6 |
| St Albans Field | SU603716 | 5.1 ± 0.0 | 10.6 ± 0.0 | 54.2 ± 1.0 | 13.2 ± 0.2 | 5 | 0 | 2 | 1 | 0 | 0 | 1 | 90 | 16.2 ± 0..1 | 2402.2 ± 3.9 | 3.9 ± 0.1 | 262.1 ± 1.8 | 72.4 ± 0.1 | 6.7 ± 0.2 | 82.3 ± 0.8 |
| St Albans Wood | SU602716 | 4.3 ± 0.0 | 90.2 ± 1.0 | 143.2 ± 2.8 | 35.1 ± 0.1 | 10 | 4 | 4 | 2 | 0 | 0 | 1 | 77 | 2.2 ± 0.0 | 5817.0 ± 45.7 | 0.2 ± 0.0 | 400.6 ± 2.6 | 292.7 ± 2.4 | 43.1 ± 0.4 | 34.9 ± 0.6 |
| Tidmarsh | SU626706 | 7.2 ± 0.0 | 5.7 ± 0.1 | 39.2 ± 0.8 | 14.7 ± 0.6 | 7 | 1 | 3 | 2 | 0 | 0 | 0 | 86 | 1.7 ± 0.2 | 3324.0 ± 487.7 | 0.1 ± 0.0 | 244.7 ± 33.6 | 57.8 ± 8.5 | 12.8 ± 1.4 | 44.0 ± 0.1 |
| Wilderness | SU738715 | 6.1 ± 0.0 | 27.6 ± 0.1 | 78.1 ± 1.4 | 42.4 ± 0.1 | 10 | 3 | 5 | 2 | 1 | 0 | 1 | 79 | 2.7 ± 0.1 | 6556.0 ± 7.8 | 0.3 ± 0.01 | 307.1 ± 0.9 | 319.7 ± 0.3 | 44.3 ± 0.3 | 15.4 ± 0.2 |

[1]These correspond to the soil series from which the sample was taken (Jarvis, 1968; Kay, 1936) except for “Soil Science” and “Wilderness” which were sampled on the University of Reading campus; [2]Obtained commercially from Broughton Loam and Turf Management, Kettering, UK; [3]Loss on ignition, %; [4]Water holding capacity, $g_{H2O}$ $g^{-1}_{soil}$; [5]Cation exchange capacity, $cmol_c$ kg$^{-1}$ (Hendershot and Duquette, 1986); [6]By X-ray fluorescence, normalised to 100 %; [7]Method of Hendershot and Duquette (1986) except P which is Olsen P (MAFF, 1986)

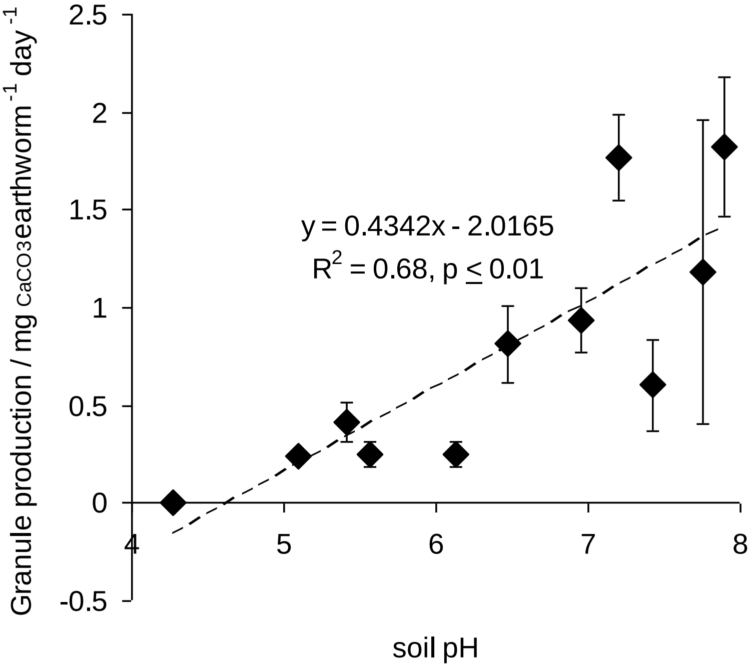


Fig. 1. Mean granule production rate for soils of different pH. Error bars = standard errors, n = 5 - 6.

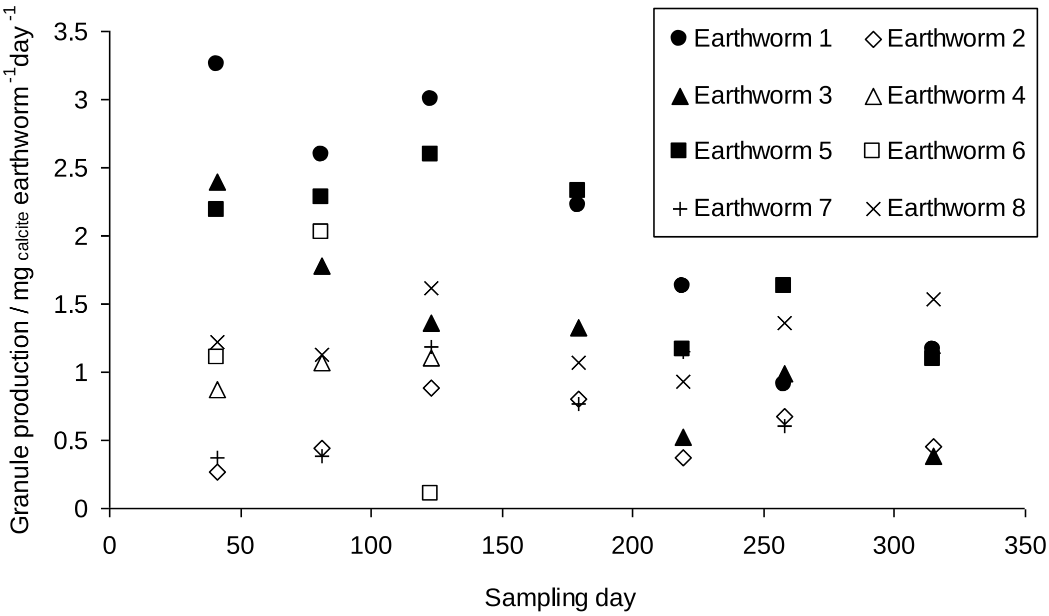


Fig. 2. Granule production rate over time in the Hamble soil. After each sampling date earthworms were put into fresh soil. Earthworms 4 and 6 died at some point between the second (Day 81) and third (Day 123) sampling dates.