

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

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

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1 ABSTRACT

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

7 1. INTRODUCTION

8 Earthworms secrete granules of calcium carbonate, predominantly calcite but also aragonite,
9 vaterite and amorphous calcium carbonate (Gago-Duport et al., 2008; Lee et al. 2008). The
10 granules are produced in the calciferous glands of the earthworm. In the case of *L. terrestris*
11 these occur in segments 10, 11 and 12 as three pairs of swellings off the oesophagus.
12 Micron-scale “spherites” of amorphous calcium carbonate are secreted in the rear two pairs of
13 oesophageal glands and move forwards into a pair of oesophageal pouches, by which time they
14 have largely crystallised to calcite and have combined to form granules. The granules are
15 secreted from the oesophageal pouches through a sphincter into the oesophagus. The function
16 that these granules serve for the earthworm is unknown (Darwin, 1881; Robertson, 1936;
17 Pearce, 1972; Briones et al., 2008) but previous studies have shown that earthworm granules
18 are commonplace in soils (Ponomareva, 1948; Wiecek and Messenger, 1972; Bal, 1977;
19 Canti, 1998). On the basis of field measurements Wiecek and Messenger (1972) estimated
20 that excreted calcium carbonate could contribute up to $11 \text{ mol}_{\text{CaCO}_3} \text{ ha}^{-1} \text{ yr}^{-1}$ to forest soils.
21 Canti and Pearce (2003) determined that granule production rates were greatest for the
22 earthworms *Lumbricus terrestris* and *L. rubellus*. Canti (2007) estimated production rates of
23 $2.2 \text{ mg}_{\text{calcite}} \text{ day}^{-1} \text{ earthworm}^{-1}$ for *L. terrestris*. The aim of the study reported here was to
24 determine how granule production rate of *L. terrestris* varied with soil properties.

26 2. METHODS

27 Eleven soils were collected, air-dried, sieved to $< 250 \mu\text{m}$ and characterised (Table 1). In all
28 production experiments one (weighed) clitellate *L. terrestris* was added to moist soil (300 g air
29 dry soil plus sufficient deionised water to raise the soil to 65 % of its water holding capacity).
30 Moisture content was kept constant throughout the experiment by addition of deionised water

1 if treatments lost weight. Treatments were kept at 18 °C under ambient light conditions. At the
2 end of each experiment the earthworm was removed and weighed prior to release. The soil
3 was wet-sieved to 500 µm to recover freshly produced granules from the soil. These were air
4 dried and weighed.

5
6 In our first experiment earthworms were exposed to the 11 different soils for 27 days with six
7 replicates per treatment. Three grammes of horse manure were added to each earthworm
8 container at the start of the experiment. In a second experiment eight earthworms were each
9 separately and repeatedly exposed to Hamble soil for periods of 39 - 57 days. After each
10 exposure period granules were extracted from the soil, earthworms were weighed and then
11 transferred to fresh Hamble soil. This process was repeated a total of seven times over 315
12 days. Three grammes of horse manure were added to each container every 14 days. Data
13 were checked for normality and equality of variance. In the first experiment treatments were
14 compared using Kruskal-Wallis one-way analysis of variance on ranks and Dunn's method
15 for pairwise comparisons. In the second experiment reported correlations are Pearson
16 correlations. All statistical analysis used SigmaStat for Windows 3.01 produced by SPSS inc.
17 In the text values are expressed as mean $\pm$ standard error.

19 3. RESULTS

20 At the end of the first experiment one earthworm had escaped or died from the Coombe
21 Complex, Kettering Loam, Neville, Park Gate and St Albans Wood soils and two from the
22 Hamble replicates. Granule production varied both within and between soils ($p \leq 0.01$) (Fig.
23 1). Within individual soils there was on average a factor of 7.7 ± 2.2 ($n = 10$, St Albans Wood
24 data discounted due to an absence of granules) times difference in masses of granules
25 produced by individual earthworms in the six replicate containers. Across all soils, granule
26 production rate varied between 0 in the pH 4.3 St Albans Wood soil (the next lowest
27 production rate was $0.05 \text{ mg}_{\text{CaCO}_3} \text{ earthworm}^{-1} \text{ day}^{-1}$ in one of the pH 6.1 Wilderness soil
28 replicates) and $4.3 \text{ mg}_{\text{calcite}} \text{ earthworm}^{-1} \text{ day}^{-1}$ (in one of the Coombe Complex soil replicates,
29 pH 7.8). Average production rate for all soils was $0.8 \pm 0.1 \text{ mg}_{\text{CaCO}_3} \text{ earthworm}^{-1} \text{ day}^{-1}$ ($n =$
30 57). Of the soil properties listed in Table 1, granule production rate was strongly correlated

1 with pH ($r^2 = 0.68$, $p \leq 0.01$) but none of the other measured soil properties.

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

14 4. DISCUSSION

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

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

4
5 An average value for earthworm density is 270 earthworms m⁻², which covers a wide range of
6 species in temperate climates (Edwards and Bohlen, 1996); though not all earthworm
7 species produce granules. Using this value, granule production rates reported here of 0.05 to
8 0.8 to 4.3 mgCaCO₃ earthworm⁻¹ day⁻¹ (minimum, average and maximum values) correspond
9 to production rates of 493, 7 884 and 42 377 molCaCO₃ ha⁻¹ yr⁻¹. A more conservative
10 estimate of 10 to 20 *L. terrestris* per m² (Briones et al. 2008) yields production rates of 18 to
11 3139 molCaCO₃ ha⁻¹ yr⁻¹ which are more similar to the estimates of Wiecek and Messenger
12 (1972) for forest soils. The precise amount of calcite produced annually by earthworms clearly
13 depends on estimates of earthworm numbers as well as soil properties; however, the
14 production rates of individual earthworms suggests to us that earthworm calcite production is
15 a potentially important source of calcite in soils.

16 17 **5. CONCLUSIONS**

18 Biological variation has a significant impact on the mass of calcium carbonate secreted by
19 earthworms in the form of granules; however, soil properties do play a role as well. The mass
20 of granules produced by earthworms is significant and they are worthy of further study to
21 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 ₂ O ₃	CaO	Fe ₂ O ₃	K ₂ O	MgO	Na ₂ O	P ₂ O 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
		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
		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
Kettering	N/A ²	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
		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
Parkgate	SU601684	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
		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
Soil Science	SU731718	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
		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
Tidmarsh	SU626706	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
		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

¹These 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; ²Obtained commercially

from Broughton Loam and Turf Management, Kettering, UK; ³Loss on ignition, %; ⁴Water holding capacity, g_{H2O} g⁻¹ soil; ⁵Cation exchange capacity, cmol_c kg⁻¹ (Hendershot and Duquette, 1986); ⁶By X-ray fluorescence, normalised to 100 %; ⁷Method of Hendershot and Duquette (1986) except P which is Olsen P (MAFF, 1986)

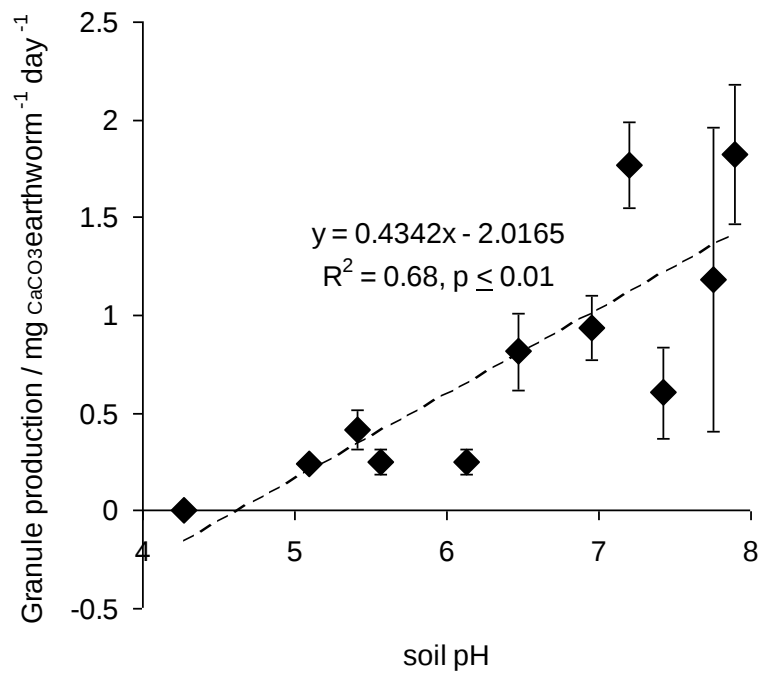


Fig. 1. Mean granule production rate for soils of different pH. Error bars = standard errors, n = 5 - 6.

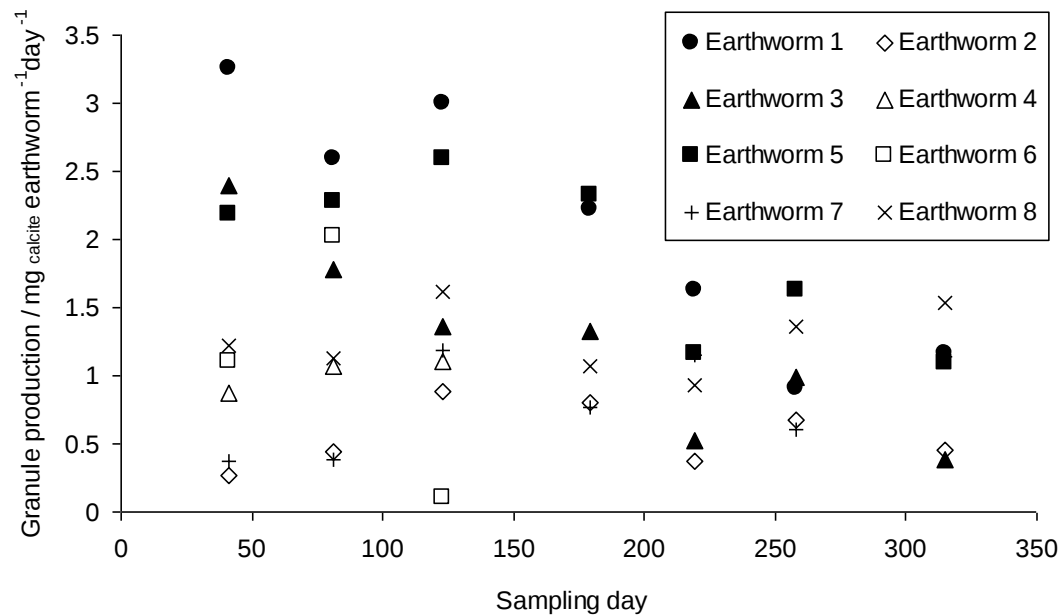


Fig. 2. Granule production rate over time in the Hamble soil. After each sampling date earthworms were put into fresh soil. Earthworms 4 and 6 died at some point between the second (Day 81) and third (Day 123) sampling dates.