

Earthworm-produced calcite granules: a new terrestrial palaeothermometer?

Article

Accepted Version

Open Access

Versteegh, E. A.A., Black, S. ORCID: <https://orcid.org/0000-0003-1396-4821>, Canti, M. G. and Hodson, M. E. (2013) Earthworm-produced calcite granules: a new terrestrial palaeothermometer? *Geochimica et Cosmochimica Acta*, 123. pp. 351-357. ISSN 0016-7037 doi: <https://doi.org/10.1016/j.gca.2013.06.020> Available at <https://centaur.reading.ac.uk/32881/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.1016/j.gca.2013.06.020>

Publisher: Elsevier for Geochemical Society and Meteoritical Society

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

Accepted Manuscript

Earthworm-produced calcite granules: a new terrestrial palaeothermometer?

Emma A.A. Versteegh, Stuart Black, Matthew G. Canti, Mark E. Hodson

PII: S0016-7037(13)00354-2

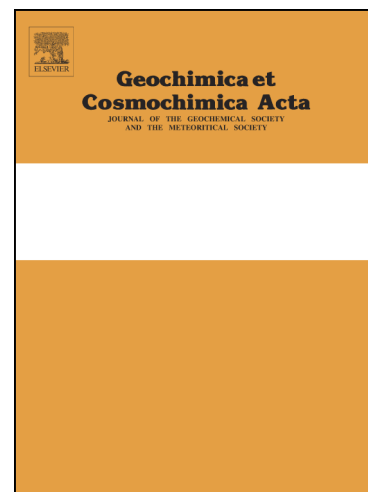
DOI: <http://dx.doi.org/10.1016/j.gca.2013.06.020>

Reference: GCA 8324

To appear in: *Geochimica et Cosmochimica Acta*

Received Date: 19 December 2012

Accepted Date: 12 June 2013



Please cite this article as: Versteegh, E.A.A., Black, S., Canti, M.G., Hodson, M.E., Earthworm-produced calcite granules: a new terrestrial palaeothermometer?, *Geochimica et Cosmochimica Acta* (2013), doi: <http://dx.doi.org/10.1016/j.gca.2013.06.020>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 **Earthworm-produced calcite granules: a new terrestrial palaeothermometer?**

2

3 Emma A. A. Versteegh^{a,*}, Stuart Black^b, Matthew G. Canti^c and Mark E. Hodson^d

4

5 ^a Department of Geography and Environmental Science, University of Reading, Whiteknights
6 P.O. Box 233, Reading RG6 6DW, UK, e.a.versteegh@reading.ac.uk

7 ^b Department of Archaeology, University of Reading, Whiteknights P.O. Box 227, Reading
8 RG6 6AB, UK, s.black@reading.ac.uk

9 ^c English Heritage, Centre for Archaeology, Fort Cumberland, Fort Cumberland Road,
10 Portsmouth PO4 9LD, UK, matthew.canti@english-heritage.org.uk

11 ^d Environment Department, University of York, Heslington, York YO10 5DD, UK,
12 mark.hodson@york.ac.uk

13 *Corresponding author. Phone: +44 (0)118 378 7896.

14

15 **Abstract**

16 In this paper we show for the first time that calcite granules, produced by the earthworm
17 *Lumbricus terrestris*, and commonly recorded at sites of archaeological interest, accurately
18 reflect temperature and soil water $\delta^{18}\text{O}$ values. Earthworms were cultivated in an orthogonal
19 combination of two different (granule-free) soils moistened by three types of mineral water
20 and kept at three temperatures (10, 16 and 20 °C) for an acclimatisation period of three weeks
21 followed by transfer to identical treatments and cultivation for a further four weeks.

22 Earthworm-secreted calcite granules were collected from the second set of soils. $\delta^{18}\text{O}$ values
23 were determined on individual calcite granules ($\delta^{18}\text{O}_c$) and the soil solution ($\delta^{18}\text{O}_w$). The
24 $\delta^{18}\text{O}_c$ values reflect soil solution $\delta^{18}\text{O}_w$ values and temperature, but are consistently enriched
25 by 1.51 (± 0.12) ‰ in comparison to equilibrium in synthetic carbonates. The data fit the

26 equation $1000 \ln \alpha = [20.21 \pm 0.92] (10^3 T^{-1}) - [38.58 \pm 3.18]$ ($R^2 = 0.95$; $n = 96$; $p < 0.0005$).

27 As the granules are abundant in modern soils, buried soils and archaeological contexts, and
28 can be dated using U-Th disequilibria, the developed palaeotemperature relationship has
29 enormous potential for application to Holocene and Pleistocene time intervals.

30

31

1. INTRODUCTION

32 Many organisms form reliable archives of palaeotemperature or water composition, by
33 precipitating calcium carbonate (CaCO_3) in oxygen isotopic equilibrium with their
34 environment (Ullmann et al., 2010; Versteegh et al., 2010; Wanamaker et al., 2007). The
35 $\delta^{18}\text{O}$ values of the CaCO_3 are controlled by temperature and water isotope composition
36 (Epstein et al., 1953; Urey, 1947), with equilibrium being defined via a mineral-specific
37 fractionation factor (Kim and O'Neil, 1997). The $\delta^{18}\text{O}$ composition of CaCO_3 produced by
38 some biomineralising taxonomic groups shows a systematic offset from equilibrium, and as
39 such is still useful as a proxy (Ford et al., 2010; Rosenheim et al., 2009; Ziveri et al., 2003).
40 The CaCO_3 minerals produced by other taxonomic groups are not in equilibrium with the
41 environment, but exhibit deviations, called vital effects (Correa et al., 2010; Juillet-Leclerc et
42 al., 2009; Owen et al., 2002). Therefore, when a biomineral is investigated as a potential
43 palaeoenvironmental proxy, it is important to develop species-specific and well-constrained
44 palaeotemperature relationships.

45 Although not widely appreciated, many earthworm species are true biomineralisers (Briones
46 et al., 2008b) and produce calcite granules in specialised glands (Canti, 1998; Lee et al.,
47 2008b) first noted by Darwin (1881; Fig. 1). The lob worm *Lumbricus terrestris* is common
48 in Europe and increasingly as an invasive species in the USA and Canada. It is one of the
49 major producers of earthworm-secreted calcite granules in temperate soils. As such a large
50 majority of these distinctly shaped granules, that are commonly found in soils, will have been

51 produced by this species (Canti, 2007). The granules are formed in the calciferous glands of
52 the earthworm, occurring in segments 10-12 as three pairs of swellings off the oesophagus
53 (Canti, 1998). The function that granule secretion serves is unknown, with suggestions
54 ranging from excretion of excess calcium as a reaction against calcium toxicity of soils, to
55 neutralisation of gut pH and regulation of CO₂ (Bal, 1977; Becze-Deák et al., 1997; Crang et
56 al., 1968; Darwin, 1881; Pearce, 1972; Robertson, 1936). Data show that granules
57 incorporate both dietary and atmospheric C (Briones et al., 2008b; Canti, 2009). They have
58 been reported from the Pleistocene (Green et al., 2006; Meijer, 1985), but can likely be
59 preserved for longer (Lambkin et al., 2011). Preliminary data suggest they can be dated using
60 U-Th disequilibria: granules recovered from Silbury Hill, a Neolithic monument in Wiltshire,
61 UK (Atkinson, 1967), which has an earliest date of 4400 BP produce a U-Th disequilibrium
62 age of 4670 ± 440 years (own data). Further U-Th analyses are ongoing and applications and
63 limitations will be discussed in a future publication. ¹⁴C dating of earthworm granules is
64 possible if enough material is available from the same stratigraphic unit and yields ages that
65 agree with other carbonates (Pustovoytov and Terhorst, 2004). Due to their abundance and
66 good preservation, calcite δ¹⁸O (δ¹⁸O_c) values from these granules form a potentially
67 powerful palaeotemperature proxy. A limited number of oxygen isotope analyses have
68 recently been published on earthworm calcite, showing similar values as directly precipitated
69 secondary carbonates, but no systematic investigation into temperature relationships has yet
70 been performed (Koeniger et al., 2012; Pustovoytov and Terhorst, 2004). Therefore, the aim
71 of the experimental work presented here was to investigate this potential by testing the
72 hypothesis that the δ¹⁸O values recorded in the earthworm secreted granules vary
73 systematically with soil solution δ¹⁸O values and temperature.

74

75

2. METHODS

76 We investigated the utility of calcite $\delta^{18}\text{O}_c$ values of the earthworm-secreted granules as a
77 palaeothermometer by means of a laboratory experiment with an orthogonal combination of
78 two different types of soil, three types of mineral water (initial $\delta^{18}\text{O}$ values -10.0, -7.3 and -
79 6.3 (± 0.2) ‰VSMOW) and three temperatures (10, 16 and 20 °C) with 6 replicates
80 (individual earthworms) per treatment.

81

82 Soils were collected from agricultural fields in Berkshire, UK: Hamble (SU 61968 70235) a
83 Hamble series Typical Argillic Brown Earth with 1.3 weight% Ca, and Red Hill (SU 56060
84 80033) a Yattendon series Typical Argillic Brown Earth with 0.6 weight% Ca (Avery, 1980;
85 Jarvis, 1968). Following our established methodology for the collection of earthworm
86 secreted granules produced over the course of an experiment, the soils were air-dried and
87 sieved to 250 μm prior to use (Lambkin et al., 2011). This ensures soils are granule-free
88 initially, and facilitates granule recovery at the end of the experiments. For each replicate,
89 300 g of soil were mixed with one of 3 different types of mineral water to 65 % water holding
90 capacity (BS ISO, 1998). This was put in a zip-lock bag with 5 g air-dried horse manure
91 rehydrated with 10 ml of the same mineral water. One adult *L. terrestris* was added to each
92 bag, and they were placed in one of three constant temperature rooms at 10, 16, or 20 °C in
93 darkness. There were 6 replicates for each treatment. A scoping study indicated that within 3
94 weeks exposure to new isotopic conditions the oxygen isotopic composition of the granules
95 had reached a steady state. Therefore earthworms were acclimatised for three weeks, and then
96 transferred to an identical treatment bag containing the same type and mass of soil, manure
97 and water at the same temperature. After 28 days earthworms were removed and soil solution
98 was extracted using rhizon samplers (Van Walt Micro Rhizon; Dickens et al., 2007; Kölling
99 et al., 2005; Seeberg-Elverfeldt et al., 2005). The samplers produce water with a water vapour
100 pressure of 2340 Pa at ambient temperature (20°C) and hence any fractionation of the water

101 isotopes during this process will be minimal. The soil was wet-sieved to 500 μm to retrieve
102 granules, which were air-dried and weighed.

103 Oxygen isotope analyses on the original mineral waters added and soil solutions extracted at
104 the end of the experiment were performed on a Picarro L2120-i Isotopic Water Analyzer with
105 an A0211 High-Precision Vaporizer and ChemCorrect software. Values were calibrated
106 against reference standards IA-R052, IA-R053 and IA-R054 from Iso-Analytical Limited.

107 Long-term reproducibility was $< 0.2 \text{ ‰}$. Nine soils did not yield enough water for analysis. In
108 order to incorporate granule data from these in our analysis we used the average water $\delta^{18}\text{O}$
109 ($\delta^{18}\text{O}_w$) value from the replicates of the same treatment. Calcite granules were individually
110 analysed for $\delta^{18}\text{O}_c$ values, using a Thermo Delta V Advantage IRMS with a GasBench II.

111 The raw $\delta^{18}\text{O}_c$ values were converted into the VPDB scale after normalising against NBS18
112 and NBS19 carbonate standards. The long-term standard deviation of a routinely analysed in-
113 house CaCO_3 standard was $< 0.1 \text{ ‰}$. Soil solution pH was measured with a combination
114 micro-electrode connected to a Hanna pH21 pH/mV meter and calibrated using pH 4.0 and
115 7.0 buffers. Ca^{2+} concentrations in soil solutions were determined using a Perkin Elmer
116 Optima 3000 ICP-OES. Operational blanks had Ca^{2+} concentrations below detection (16
117 $\mu\text{g/l}$). Data were calibrated via analysis of 1, 50 and 100 ppm dilutions of the Merck ICP
118 multi-element standard solution IV and accuracy determined through analysis of an in house
119 standard.

121 3. RESULTS

122 Results are shown in Table 1 and Fig. 2. At the end of the 4 week exposure, soil solution
123 $\delta^{18}\text{O}_w$ values ranged from -10.2 to -5.3 ‰ VSMOW . Some influence of evaporation was
124 observed, causing higher $\delta^{18}\text{O}_w$ values for higher temperatures. This is most pronounced for
125 the Hamble soil, probably because of its lower water holding capacity (Table 1, Fig. 2). $\delta^{18}\text{O}_c$

126 values of individual granules ($n = 931$) vary between -10.10 and -3.21 ‰VPDB. Within-
 127 treatment $\delta^{18}\text{O}_c$ values of granules are normally distributed. The variability in $\delta^{18}\text{O}_c$ values
 128 between granules produced by an individual earthworm within a single month is on average
 129 0.61 ‰ (range 1.44 ‰). Higher soil solution $\delta^{18}\text{O}_w$ values yield higher $\delta^{18}\text{O}_c$ values, and
 130 higher temperatures result in lower $\delta^{18}\text{O}_c$ values (Fig. 2).

131

132

4. DISCUSSION

133 For each replicate the expected $\delta^{18}\text{O}_c$ value for equilibrium was calculated from the $\delta^{18}\text{O}_w$
 134 value and temperature according to the equation of Kim and O'Neil (1997):

135

$$136 \quad 1000 \ln \alpha = 18.03 (10^3 T^{-1}) - 32.42 \quad (1)$$

137

138 The resulting predicted $\delta^{18}\text{O}_c$ ($\delta^{18}\text{O}_{\text{pred}}$) values are plotted with average measured $\delta^{18}\text{O}_c$ per
 139 replicate in Figure 3. A linear regression shows that earthworm granules are systematically
 140 enriched in ^{18}O by 1.51 (± 0.12 s.d.) ‰ in comparison to equilibrium:

141

$$142 \quad \delta^{18}\text{O}_c = 1.02 \delta^{18}\text{O}_{\text{pred}} + 1.51 \quad (2)$$

143

144 with $R^2 = 0.98$; $n = 96$; $p < 0.0005$.

145 Subsequently, the fractionation factor α was derived:

146

$$147 \quad \alpha_{\text{calcite-water}} = ([1000 + \delta^{18}\text{O}_c \text{VSMOW}] / [1000 + \delta^{18}\text{O}_w \text{VSMOW}]) \quad (3)$$

148

149 and plotted against $10^3 T^{-1}$ (K). Regression analysis yields the palaeotemperature relationship:

150

151 $1000 \ln \alpha = [20.21 \pm 0.92] (10^3 T^{-1}) - [38.58 \pm 3.18]$ (4)

152

153 with $R^2 = 0.95$; $n = 96$; $p < 0.0005$. Quoted errors on the slope and intercept are reported at
154 the 95 % confidence interval. Our results are compared to equations by Kim & O'Neil (1997;
155 synthetic carbonates) and Wanamaker et al. (2006; 2007; Blue mussel, *Mytilus edulis*) in
156 Figure 4. *M. edulis* data are slightly offset from equilibrium (< 0.2 ‰), but this difference is
157 not statistically significant (Wanamaker et al., 2007). $\delta^{18}\text{O}_c$ values of calcite granules
158 produced by *L. terrestris* reflect soil solution $\delta^{18}\text{O}_w$ values and temperature, but are enriched
159 in ^{18}O in comparison to equilibrium in synthetic carbonates (Kim and O'Neil, 1997) and the
160 bivalve *M. edulis* (Wanamaker et al., 2007).

161

162 Although the relationship is strong, the range of $1000 \ln \alpha$ for a given temperature is about
163 twice that observed for *M. edulis* (Wanamaker et al., 2007) translating into an average offset
164 in reconstructed temperature of 0.73 °C. A possible explanation for this increased variation is
165 that some evaporation occurred during the experiment, progressively increasing soil solution
166 $\delta^{18}\text{O}_w$ values throughout the 4-week experiment. In addition, some of the variation could
167 have been caused by the variability of the controlled temperature chambers in which the
168 experiments were performed at ± 0.5 °C from the set temperature.

169

170 The enrichment in ^{18}O by 1.51 ‰ compared to equilibrium is statistically indistinguishable
171 from the equation for synthetic calcite precipitated from a solution with initial $[\text{Ca}^{2+}]$ and
172 $[\text{HCO}_3^-]$ of 15 mM (Fig. 4; Kim and O'Neil, 1997). The soil solutions collected after the
173 experiment had $[\text{Ca}^{2+}]$ values varying between $8.30 (\pm 1.02 \text{ s.d.})$ mM and $16.79 (\pm 1.42 \text{ s.d.})$
174 mM with higher values for higher temperatures, for mineral waters with higher initial $[\text{Ca}^{2+}]$,
175 and for the Red Hill soil. Average pH values were $6.5 \pm 0.8 \text{ s.d.}$ (Fiji), $6.8 \pm 0.5 \text{ s.d.}$

176 (England) and 7.0 ± 0.3 s.d. (Norway; Table 1). pH did not significantly differ between
177 temperatures or soils. At the measured pH values, most DIC will be present in the form of
178 HCO_3^- (Zeebe and Wolf-Gladrow, 2001). HCO_3^- was not determined directly, but
179 concentrations of dissolved inorganic carbon (DIC) are likely to have been high (up to 32
180 mM based on equivalence of charge of DIC with Ca^{2+}), due to earthworm and microorganism
181 respiration ($601\text{-}1329 \mu\text{mol/mol CO}_2$ in soil air, own data). As such, it appears that elevated
182 initial $[\text{Ca}^{2+}]$ and $[\text{HCO}_3^-]$ caused the ^{18}O enrichment in earthworm granules, through the
183 same unknown mechanism as in synthetic calcite. Earthworms do not produce calcite
184 granules at low $[\text{Ca}^{2+}]$ (own data) or low pH (Lambkin et al., 2011), and thus the range of
185 these parameters in soils containing granules will be constrained at the lower end. Therefore,
186 different soils are unlikely to yield radically different palaeotemperature equations.

187
188 A possible alternative or additional explanation for the 1.51 ‰ offset of the granules lies in
189 precipitation kinetics. Calcite precipitation in the calciferous gland likely takes place along a
190 pathway of dissolved CO_2 transforming to HCO_3^- and CO_3^{2-} , then to amorphous CaCO_3 ,
191 which finally stabilises into calcite through a dissolution-reprecipitation mechanism (Briones
192 et al., 2008a; Lee et al., 2008a). If calcite precipitation were very fast, there would be
193 incomplete fractionation between HCO_3^- and CaCO_3 , resulting in ^{18}O enrichment of
194 earthworm granules in comparison to equilibrium (kinetic effect) (Lachniet, 2009; Mickler et
195 al., 2004). The precipitation rate of calcite varied between $1.24 (\pm 0.47 \text{ s.d.})$ and $2.89 (\pm 0.31$
196 $\text{ s.d.}) \text{ mg/day}$ and was higher for higher temperatures and the Red Hill soil, and lower for
197 Norway water (Table 1). However, no relationship between precipitation rate and $\delta^{18}\text{O}_c$
198 values was found.

199

200 Several recent publications on speleothems and inorganically precipitated calcite suggest that
201 $\alpha_{\text{calcite-water}}$ is greater than the commonly accepted value by up to 1.5 ‰ (Coplen, 2007; Day
202 and Henderson, 2011; Dietzel et al., 2009; Feng et al., 2012; Tremaine et al., 2011). Although
203 these systems are very different from the earthworm calciferous gland, it is possible, that they
204 have a common cause for disequilibrium, such as fractionation reactions at the crystal
205 surface.

206

207 For application in palaeotemperature reconstructions, a good estimate of soil water $\delta^{18}\text{O}_w$
208 values is needed. Due to the combined influence of seasonal variations in precipitation $\delta^{18}\text{O}_w$
209 values and evapotranspiration, these vary over time and with depth (Hsieh et al., 1998).

210 However, in a high-rainfall temperate climate, like that of the British Isles, the influence of
211 evaporation is likely to be small. This is supported by data showing that the $\delta^{18}\text{O}_w$

212 composition of groundwater reflects that of local precipitation within 0.5 ‰ (Darling et al.,
213 2003) and that $\delta^{18}\text{O}_c$ values of modern soil carbonate are correlated with $\delta^{18}\text{O}_w$ values of

214 local meteoric water (Cerling, 1984). For the past 4000 years, modern values for the isotopic
215 composition of precipitation can be used (McDermott et al., 2011). For earlier Holocene and

216 Pleistocene time intervals, $\delta^{18}\text{O}_w$ values of precipitation are often not well-constrained and

217 the earthworm-calcite palaeothermometer can only be used if an independent reconstruction

218 of soil water $\delta^{18}\text{O}_w$ values is available. Alternatively, the equation can serve as an

219 independent proxy for soil water $\delta^{18}\text{O}_w$ values (and hence $\delta^{18}\text{O}_w$ values of precipitation) in

220 the presence of existing palaeotemperature reconstructions.

221

222

5. CONCLUSIONS

223 In summary, our study shows that the relationship between temperature and earthworm

224 calcite $\delta^{18}\text{O}_c$ values is significant and predictable. In combination with the wide distribution,

225 good preservation, and direct U-Th dating of the granules, these will make a novel terrestrial
226 temperature proxy. Important questions to be addressed are if this relationship holds under
227 field conditions when evaporation of soil moisture may occur, and for other earthworm
228 species. Future work will investigate these issues and also include application of the
229 developed palaeothermometer to granules retrieved from various Holocene and Pleistocene
230 locations and archaeological sites in Great Britain, the Netherlands and Germany.

231

232

ACKNOWLEDGEMENTS

233 This research was funded by a NERC Standard Research Grant (M.E.H. and S.B. ;
234 NE/H021914/1). We would like to thank Yan Gao for assistance with stable isotope analyses,
235 Anne Dudley and Martin Heaps for help with the Ca analyses, and Alan Wanamaker for
236 making available his *M. edulis* data. In addition, we thank Frank McDermott, Tim Atkinson
237 and an anonymous reviewer for constructive comments that were of great help in improving
238 the manuscript.

239

240

REFERENCES

- 241 Atkinson R.J.C. (1967) Silbury Hill. *Antiquity* **XLI**, 259-262.
- 242 Avery B.W. (1980) *Soil classification for England and Wales [higher categories]*.
243 Rothamsted Experimental Station, Harpenden.
- 244 Bal L. (1977) The formation of carbonate nodules and intercalary crystals in the soil by the
245 earthworm *Lumbricus rubellus*. *Pedobiologia* **17**, 229-240.
- 246 Becze-Deák J., Langohr R., Verrecchia E.P. (1997) Small scale secondary CaCO₃
247 accumulations in selected sections of the European loess belt. Morphological forms
248 and potential for paleoenvironmental reconstruction. *Geoderma* **76**, 221-252.

- 249 Briones M.J.I., López E., Méndez J., Rodríguez J.B., Gago-Duport L. (2008a) Biological
250 control over the formation and storage of amorphous calcium carbonate by
251 earthworms. *Mineral. Mag.* **72**, 227-231.
- 252 Briones M.J.I., Ostle N.J., Pearce T.G. (2008b) Stable isotopes reveal that the calciferous
253 gland of earthworms is a CO₂-fixing organ. *Soil Biol. Biochem.* **40**, 554-557.
- 254 BS ISO (1998) *Soil quality - Determination of the water-retention characteristic*. BS ISO,
255 Geneva.
- 256 Canti M.G. (1998) Origin of calcium carbonate granules found in buried soils and Quaternary
257 deposits. *Boreas* **27**, 275-288.
- 258 Canti M.G. (2007) Deposition and taphonomy of earthworm granules in relation to their
259 interpretative potential in Quaternary stratigraphy. *J. Quatern. Sci.* **22**, 111-118.
- 260 Canti M.G. (2009) Experiments on the origin of ¹³C in the calcium carbonate granules
261 produced by the earthworm *Lumbricus terrestris*. *Soil Biol. Biochem.* **41**, 2588-2592.
- 262 Cerling T.E. (1984) The stable isotopic composition of modern soil carbonate and its
263 relationship to climate. *Earth Planet. Sci. Lett.* **71**, 229-240.
- 264 Coplen T.B. (2007) Calibration of the calcite–water oxygen-isotope geothermometer at
265 Devils Hole, Nevada, a natural laboratory. *Geochim. Cosmochim. Acta* **71**, 3948-
266 3957.
- 267 Correa M.L., Montagna P., Vendrell-Simón B., McCulloch M., Taviani M. (2010) Stable
268 isotopes ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$), trace and minor element compositions of recent
269 scleractinians and Last Glacial bivalves at the Santa Maria di Leuca deep-water coral
270 province, Ionian Sea. *DSR* **57**, 471-486.
- 271 Crang R.E., Holsen R.C., Hitt J.B. (1968) Calcite Production in Mitochondria of Earthworm
272 Calciferous Glands. *Bioscience* **18**, 299-301.

- 273 Darling W.G., Bath A.H., Talbot J.C. (2003) The O and H stable isotope composition of
274 freshwaters in the British Isles. 2. Surface waters and groundwater. *HESS* **7**, 183-195.
- 275 Darwin C. (1881) *The formation of vegetable mould, through the action of worms, with*
276 *observations on their habits*. The Echo Library, Teddington.
- 277 Day C.C., Henderson G.M. (2011) Oxygen isotopes in calcite grown under cave-analogue
278 conditions. *Geochim. Cosmochim. Acta* **75**, 3956-3972.
- 279 Dickens G.R., Koelling M., Smith D.C., Schnieders L. (2007) Rhizon sampling of pore
280 waters on scientific drilling expeditions: an example from the IODP Expedition 302,
281 Arctic Coring Expedition (ACEX). *Scientific Drilling* **4**, 22-25.
- 282 Dietzel M., Tang J., Leis A., Köhler S.J. (2009) Oxygen isotopic fractionation during
283 inorganic calcite precipitation — Effects of temperature, precipitation rate and pH.
284 *Chem. Geol.* **268**, 107-115.
- 285 Epstein S., Buchsbaum R., Lowenstam H.A., Urey H.C. (1953) Revised carbonate-water
286 isotopic temperature scale. *Bulletin of the Geological Society of America* **64**, 1315-
287 1326.
- 288 Feng W., Banner J.L., Guilfoyle A.L., Musgrove M., James E.W. (2012) Oxygen isotopic
289 fractionation between drip water and speleothem calcite: A 10-year monitoring study,
290 central Texas, USA. *Chem. Geol.* **304–305**, 53-67.
- 291 Ford H.L., Schellenberg S.A., Becker B.J., Deutschman D.L., Dyck K.A., Koch P.L. (2010)
292 Evaluating the skeletal chemistry of *Mytilus californianus* as a temperature proxy:
293 Effects of microenvironment and ontogeny. *Paleoceanography* **25**.
- 294 Green C.P., Branch N.P., Russell Coope G., Field M.H., Keen D.H., Wells J.M.,
295 Schwenninger J.-L., Preece R.C., Schreve D.C., Canti M.G., Gleed-Owen C.P. (2006)
296 Marine Isotope Stage 9 environments of fluvial deposits at Hackney, north London,
297 UK. *Quatern. Sci. Rev.* **25**, 89-113.

- 298 Hsieh J.C.C., Chadwick O.A., Kelly E.F., Savin S.M. (1998) Oxygen isotopic composition of
299 soil water: Quantifying evaporation and transpiration. *Geoderma* **82**, 269-293.
- 300 Jarvis R.A. (1968) *Soils of the Reading district*. Agricultural Research Council, Harpenden.
- 301 Juillet-Leclerc A., Reynaud S., Rollion-Bard C., Cuif J.P., Dauphin Y., Blamart D., Ferrier-
302 Pagès C., Allemand D. (2009) Oxygen isotopic signature of the skeletal
303 microstructures in cultured corals: Identification of vital effects. *Geochim.*
304 *Cosmochim. Acta* **73**, 5320-5332.
- 305 Kim S.-T., O'Neil J.R. (1997) Equilibrium and nonequilibrium oxygen isotope effects in
306 synthetic carbonates. *Geochim. Cosmochim. Acta* **61**, 3461-3475.
- 307 Koeniger P., Barta G., Thiel C., Bajnóczi B., Novothny Á., Horváth E., Techmer A., Frechen
308 M. (2012) Stable isotope composition of bulk and secondary carbonates from the
309 Quaternary loess-paleosol sequence in Süttő, Hungary. *Quatern. Int.*
- 310 Kölling M., Seeberg-Elverfeldt J., Schlüter M. (2005) Rhizon-an excellent pore water
311 sampler for low maintenance collection and filtration of small volume samples. In (
- 312 Lachniet M.S. (2009) Climatic and environmental controls on speleothem oxygen-isotope
313 values. *Quatern. Sci. Rev.* **28**, 412-432.
- 314 Lambkin D.C., Gwilliam K.H., Layton C., Canti M.G., Pearce T.G., Hodson M.E. (2011)
315 Production and dissolution rates of earthworm-secreted calcium carbonate.
316 *Pedobiologia* **54**, S119-S129.
- 317 Lee M.R., Hodson M.E., Langworthy G.N. (2008a) Crystallization of calcite from amorphous
318 calcium carbonate: earthworms show the way. *Mineral. Mag.* **72**, 257-261.
- 319 Lee M.R., Hodson M.E., Langworthy G.N. (2008b) Earthworms produce granules of
320 intricately zoned calcite. *Geology* **36**, 943-946.

- 321 McDermott F., Atkinson T.C., Fairchild I.J., Baldini L.M., Matthey D.P. (2011) A first
322 evaluation of the spatial gradients in $\delta^{18}\text{O}$ recorded by European Holocene
323 speleothems. *Global Planet. Change* **79**, 275-287.
- 324 Meijer T. (1985) The pre-Weichselian non-marine molluscan fauna from Maastricht-
325 Belvédère (Southern Limburg, The Netherlands). In *Maastricht-Belvédère:
326 Stratigraphy, palaeoenvironment and archaeology of the Middle and Late Pleistocene
327 depositis* (Geological Survey of The Netherlands, Haarlem, pp. 75-103.
- 328 Mickler P.J., Banner J.L., Stern L., Asmerom Y., Edwards R.L., Ito E. (2004) Stable isotope
329 variations in modern tropical speleothems: Evaluating equilibrium vs. kinetic isotope
330 effects. *Geochim. Cosmochim. Acta* **68**, 4381-4393.
- 331 Owen R., Kennedy H., Richardson C. (2002) Isotopic partitioning between scallop shell
332 calcite and seawater: effect of shell growth rate. *Geochim. Cosmochim. Acta* **66**,
333 1727-1737.
- 334 Pearce T.G. (1972) The Calcium Relations of Selected Lumbricidae. *J. Anim. Ecol.* **41**, 167-
335 188.
- 336 Pustovoytov K., Terhorst B. (2004) An isotopic study of a late Quaternary loess-paleosol
337 sequence in SW Germany. *Revista Mexicana de Ciencias Geológicas* **21**, 88-93.
- 338 Robertson J.D. (1936) The function of the calciferous glands of earthworms. *J. Exp. Biol.*
339 **XIII**, 279-297.
- 340 Rosenheim B.E., Swart P.K., Willenz P. (2009) Calibration of sclerosponge oxygen isotope
341 records to temperature using high-resolution $\delta^{18}\text{O}$ data. *Geochim. Cosmochim. Acta*
342 **73**, 5308-5319.
- 343 Seeberg-Elverfeldt J., Schlüter M., Feseker T., Kölling M. (2005) Rhizon sampling of pore
344 waters near the sediment/water interface of aquatic systems. *Limnol. Oceanogr.*
345 *Methods* **3**, 361-371.

- 346 Tremaine D.M., Froelich P.N., Wang Y. (2011) Speleothem calcite formed in situ: Modern
347 calibration of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ paleoclimate proxies in a continuously-monitored natural
348 cave system. *Geochim. Cosmochim. Acta* **75**, 4929-4950.
- 349 Ullmann C.V., Wiechert U., Korte C. (2010) Oxygen isotope fluctuations in a modern North
350 Sea oyster (*Crassostrea gigas*) compared with annual variations in seawater
351 temperature: Implications for palaeoclimate studies. *Chem. Geol.* **277**, 160-166.
- 352 Urey H.C. (1947) The thermodynamic properties of isotopic substances. *Journal of the*
353 *Chemical Society (Resumed)*, 562-581.
- 354 Versteegh E.A.A., Vonhof H.B., Troelstra S.R., Kaandorp R.J.G., Kroon D. (2010)
355 Seasonally resolved growth of freshwater bivalves determined by oxygen and carbon
356 isotope shell chemistry. *GGG* **11**, 16.
- 357 Wanamaker A.D., Jr., Kreutz K.J., Borns H.W., Jr., Introne D.S., Feindel S., Barber B.J.
358 (2006) An aquaculture-based method for calibrated bivalve isotope
359 paleothermometry. *GGG* **7**, 13.
- 360 Wanamaker A.D., Jr., Kreutz K.J., Borns Jr. H.W., Introne D.S., Feindel S., Funder S.,
361 Rawson P.D., Barber B.J. (2007) Experimental determination of salinity, temperature,
362 growth, and metabolic effects on shell isotope chemistry of *Mytilus edulis* collected
363 from Maine and Greenland. *Paleoceanography* **22**, 12.
- 364 Zeebe R.E., Wolf-Gladrow D. (2001) *CO₂ in seawater: equilibrium, kinetics, isotopes*.
365 Elsevier, Amsterdam.
- 366 Ziveri P., Stoll H., Probert I., Klaas C., Geisen M., Ganssen G., Young J. (2003) Stable
367 isotope 'vital effects' in coccolith calcite. *Earth Planet. Sci. Lett.* **210**, 137-149.
368
369

370 **Figure and Table legends**

371

372 Table 1. Experimental conditions. Average soil solution pH, $[\text{Ca}^{2+}]$, CaCO_3 production and
373 $\delta^{18}\text{O}_w$ values are all means \pm SD.

374

375 Figure 1. SEM pictures of a *L. terrestris*-produced calcite granule. (a) entire granule, (b)
376 surface calcite crystal structure, (c) individual calcite crystal. Images are back-scattered
377 electron images taken using a FEI Quanta FEG 600 Environmental Scanning Electron
378 Microscope. (a) and (b) are taken from Lambkin et al. (2011).

379

380 Figure 2. $\delta^{18}\text{O}_c$ values of individual granules and soil solution $\delta^{18}\text{O}_w$ values. Granules
381 produced at 10 °C (blue), 16 °C (purple) and 20 °C (red) in Hamble soil (triangles) and Red
382 Hill soil (circles). Higher $\delta^{18}\text{O}_w$ values result in higher $\delta^{18}\text{O}_c$ values and higher temperatures
383 give lower $\delta^{18}\text{O}_c$ values.

384

385 Figure 3. Comparison of $\delta^{18}\text{O}_{\text{pred}}$ values according to Kim and O'Neil (1997) and measured
386 $\delta^{18}\text{O}_c$ values for earthworm-produced calcite. *L. terrestris* CaCO_3 granules are enriched in
387 ^{18}O by 1.51 ‰.

388

389 Figure 4. Relationship between $10^3 \ln \alpha$ and temperature for several types of calcite. *L.*
390 *terrestris* granules (red diamonds; this study), equilibrium (grey solid line), synthetic at initial
391 $[\text{Ca}^{2+}]$ and $[\text{HCO}_3^-]$ of 15 mM (black), synthetic at initial $[\text{Ca}^{2+}]$ and $[\text{HCO}_3^-]$ of 25 mM (grey
392 dashed line) (Kim and O'Neil (1997)), and *M. edulis* shells (blue triangles) (Wanamaker et al.
393 (2007)). Earthworm granules are enriched in ^{18}O compared to equilibrium, in a similar way as
394 synthetic carbonates precipitated at elevated initial concentrations of Ca^{2+} and HCO_3^- .

Table 1. Experimental conditions.

T (°C)	Soil	Mineral water added	Water holding capacity (%)	Soil solution pH	Soil solution [Ca ²⁺] (mM)	CaCO ₃ production per earthworm (mg/day)	δ ¹⁸ O _w (‰ VSMOW)	N*
		Fiji		7.8	0.46		-6.3 ±0.2	1
	Pure mineral waters	England		7.4	1.20		-7.3 ±0.2	1
		Norway		7.7	0.18		-10.0 ±0.2	1
10	Hamble	Fiji	33	7.2 ±0.4	10.93 ±0.89	1.45 ±0.28	-5.93 ±0.05	5
10	Hamble	England	33	6.7 ±0.9	10.44 ±0.62	1.66 ±0.43	-7.11 ±0.04	6
10	Hamble	Norway	33	5.5 ±1.0	8.30 ±1.02	1.24 ±0.47	-9.60 ±0.05	6
10	Red Hill	Fiji	56	6.9 ±0.1	15.86 ±2.09	1.82 ±0.21	-6.04 ±0.11	6
10	Red Hill	England	56	6.7 ±0.1	13.30 ±0.82	1.77 ±0.26	-7.24 ±0.03	6
10	Red Hill	Norway	56	6.9 ±0.1	15.21 ±0.92	1.76 ±0.46	-9.85 ±0.06	6
16	Hamble	Fiji	33	7.3 ±0.2	12.13 ±1.52	2.30 ±0.24	-5.72 ±0.14	5
16	Hamble	England	33	7.1 ±0.2	11.22 ±1.31	2.11 ±0.25	-7.10 ±0.08	6
16	Hamble	Norway	33	6.7 ±0.8	9.09 ±0.87	1.79 ±0.37	-9.44 ±0.10	6
16	Red Hill	Fiji	56	6.9 ±0.1	15.82 ±0.84	2.56 ±0.27	-6.20 ±0.12	5
16	Red Hill	England	56	6.7 ±0.2	13.44 ±1.45	2.75 ±0.27	-7.24 ±0.09	6
16	Red Hill	Norway	56	6.7 ±0.2	15.25 ±0.49	2.19 ±0.49	-9.94 ±0.10	6
20	Hamble	Fiji	33	7.0 ±0.4	12.34 ±1.35	2.39 ±0.47	-5.43 ±0.10	6
20	Hamble	England	33	6.9 ±0.7	11.39 ±0.58	2.06 ±0.60	-6.74 ±0.04	6
20	Hamble	Norway	33	5.7 ±0.5	11.38 ±0.43	1.92 ±0.47	-9.02 ±0.11	5
20	Red Hill	Fiji	56	6.8 ±0.2	16.79 ±1.42	2.46 ±0.27	-5.85 ±0.05	6
20	Red Hill	England	56	6.8 ±0.3	13.81 ±0.53	2.89 ±0.31	-7.02 ±0.05	5
20	Red Hill	Norway	56	6.9 ±0.2	15.10 ±0.89	2.70 ±0.53	-9.60 ±0.10	4

* N is number of replicates per treatment. Average soil solution pH, [Ca²⁺], CaCO₃

production and δ¹⁸O_w values are all means ± s.d.

