

Why is amorphous calcium carbonate secreted by earthworms so stable?

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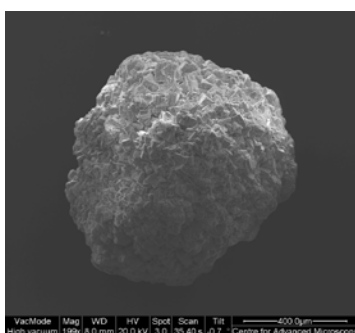


Fig. 1. SEM image of a *L. terrestris*-produced calcite grant. Back-scattered electron image taken using a FEI Quanta FEG 600 Environmental SEM [10].

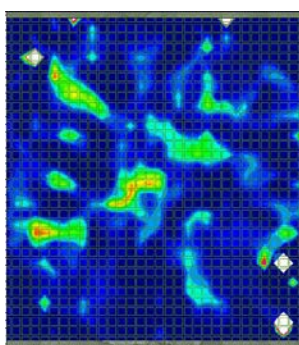


Fig. 2. v_2/v_4 ratio map of a slice through a granule with high intensity areas indicative of ACC. Image taken on the B22 beamline.

Several species of earthworm secrete millimetre-scale balls of calcium carbonate¹ (Fig. 1). The function of the secretions is not clear but seems likely to involve pH regulation within the earthworm². The granules are mainly calcite but vaterite, aragonite and amorphous calcium carbonate (ACC) have also been detected using electron back scattered diffraction, X-ray diffraction, X-ray spectroscopy and FTIR spectroscopy^{1,3-7}. The ACC appears to be unusually stable. Inorganically synthesised ACC is stable for minutes to hours⁸; the ACC in the granules is present several years after granule formation. Studies of various biological ACCs

and inorganic experiments suggest that ACC can be stabilised by inorganic components including Mg and P and a variety of organic macromolecules including polysaccharides and proteins rich in the amino acids glutamic acid, serine and glycine⁹. The mechanism by which the ACC in earthworm granules is stabilised is not known. We compared bulk ACC contents of granules with bulk elemental and amino-acid content but saw no significant relationships, most likely due to the heterogeneity of individual granules. We used Beamline B22 (Multimode InfraRed Imaging and Microspectroscopy, MIRIAM) at the Diamond Light Source to try to map the spatial distribution of ACC and organic molecules in the granules. Elemental distributions were determined using a Cameca SX100 electron microprobe. We were able to map the spatial distribution of ACC using wave number intensity, wave number ratios (Fig. 2), cluster analysis and component regression but were unsuccessful with the organic molecules, due to peak overlaps and, we think concentration levels. We saw no spatial correlation between ACC and elemental composition.

References

1. Lee, M. R., Hodson, M. E. *et al.* Earthworms produce granules of intricately zoned calcite, *Geology* **36**, 943 (2008).
2. Versteegh, E. A. A., Black, S. *et al.* Environmental controls on the production of calcium carbonate by earthworms. *Soil Biol. Biochem.* **70**, 159 (2014).
3. Brinza, L., Quinn, P. D. *et al.* Incorporation of strontium in earthworm-secreted calcium carbonate granules produced in strontium-amended and strontium-bearing soil. *Geochim. Cosmochim. Acta* **113**, 21 (2013).
4. Brinza, L., Schofield, PF *et al.* Could earthworm-secreted calcium carbonate incorporate Zn in contaminated soils? *Soil Biol. Biochem.* **74**, 1 (2014).
5. Brinza, L., Schofield, PF, *et al.* Combining μ XANES and μ XRD mapping to analyse the heterogeneity in calcium carbonate granules excreted by the earthworm *Lumbricus terrestris*. *J. Synchrotron Rad.* **21**, 235 (2014).
6. Fraser A., Lambkin, D. C., *et al.* Incorporation of lead into calcium carbonate granules secreted by earthworms living in lead contaminated soils. *Geochim. Cosmochim. Acta* **75**, 2544 (2011).
7. Gago-Duport, L., Briones, M. J. I. *et al.* Amorphous calcium carbonate biomineralization in the earthworm's calciferous gland: Pathways to the formation of crystalline phases, *J. Structural Biol.* **162**, 422 (2008).
8. Rodriguez-Blanco, J. D., Shaw, S. *et al.* The kinetics and mechanisms of Amorphous Calcium Carbonate (ACC) crystallization to calcite, via vaterite. *Nanoscale* **3**, 265 (2011).
9. Lam, R. S. K., Charnock, J. M. *et al.* Synthesis-dependant structural variations in amorphous calcium carbonate. *CrystEngComm* **9**, 1226 (2007).
10. Lambkin, D. C., Gwilliam, K. H. *et al.* Production and dissolution rates of earthworm-secreted calcium carbonate. *Pedobiol.* **54S**, S119 (2011).