LETTERS

Biomarker evidence for green and purple sulphur bacteria in a stratified Palaeoproterozoic sea

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The disappearance of iron formations from the geological record ~1.8 billion years (Gyr) ago was the consequence of rising oxygen levels in the atmosphere starting 2.45–2.32 Gyr ago[1–3]. It marks the end of a 2.5-Gyr period dominated by anoxic and iron-rich deep oceans. However, despite rising oxygen levels and a concomitant increase in marine sulphate concentration, related to enhanced sulphide oxidation during continental weathering[4], the chemistry of the oceans in the following mid-Proterozoic interval (~1.8–0.8 Gyr ago) probably did not yet resemble our oxygen-rich modern oceans. Recent data[5–8] indicate that marine oxygen and sulphate concentrations may have remained well below current levels during this period, with one model indicating that anoxic and sulphidic marine basins were widespread, and perhaps even globally distributed[4]. Here we present hydrocarbon biomarkers (molecular fossils) from a 1.64-Gyr-old basin in northern Australia, revealing the ecological structure of mid-Proterozoic marine communities. The biomarkers signify a marine basin with anoxic, sulphidic, sulphate-poor and permanently stratified deep waters, hostile to eukaryotic algae. Phototrophic purple sulphur bacteria (Chromatiaceae) were detected in the geological record based on the new carotenoid biomarker okenane, and they seem to have co-existed with communities of green sulphur bacteria (Chlorobiaceae). Collectively, the biomarkers support mounting evidence for a long-lasting Proterozoic world in which oxygen levels remained well below modern levels.

The 1.640 ± 0.003-Gyr-old[9] Barney Creek Formation (BCF) of the McArthur Group, northern Australia, was deposited in a north–south-trending rift west of the present Gulf of Carpentaria over a known area of 25,000 km^2. The BCF represents a marine succession that accumulated in a quiet, sub-wave-base environment[5,10–12]. The dominant lithologies are thinly bedded or planar laminated, dolomitic, carbonaceous and pyritic siltstones and shales, with a typical organic carbon content of 0.2–2%, and locally up to 6%[13]. Samples were collected from 12 drill holes (Supplementary Table S1) representing almost the entire north–south axis of the preserved basin. Biomarkers were extracted, fractionated and analysed by gas chromatography–mass spectroscopy in accordance with procedures described in Supplementary Material.

The bitumens of the BCF contain some of the oldest preserved, unambiguously syngenetic biomarkers known so far[14]. The distribution of biomarkers in the 48 samples analysed here shows slight variations with lithology and depositional depth but is generally distinct from distributions currently known from all other periods in Earth's history. Figure 1a shows a chromatogram of the most abundant saturated hydrocarbons in a representative dolomitic shale from the Glyde River region. Although signs of biodegradation are absent, it is dominated (more than 90% by mass) by a 'hump' or unresolved complex mixture. Regular acyclic isoprenoids with 15–20 carbon atoms are abundant and probably mainly derived from the isoprenoid side chain of (bacterio) chlorophylls. Regular acyclic isoprenoids with 21–25 carbon atoms are present in some samples and are attributed to archaea. Detected for the first time in the Precambrian were the C_{40} carotenoids lycopane, β-carotane and the tentatively identified γ-carotane. Biological precursors of these biomarkers are common in many phototrophic organisms from all environments, but they are typically preserved as hydrocarbon markers under strongly reducing conditions only.

Another unusual feature is the high relative concentration of 3β-methylhopanes (Fig. 1b), triterpanes commonly attributed to microaerophilic proteobacteria, particularly type I methanotrophs[15]. The 3β-methylhopane index for the C_{31} homologue (C_{31}-3β-MHI), defined as the abundance of the C_{31} methyl hopane relative to the corresponding C_{30} desmethyl hopane, averages 5.7% in the BCF ($n = 9$; drill core GR-7); modal values observed in typical Neoproterozoic bitumens and oils are much lower (1–2%). The high 3β-MHI indicates a very high abundance and, presumably, a high activity of type I methanotrophs. Similar conditions were observed in Phanerozoic (0.542 Gyr to present) sediments deposited in sulphate-depleted environments[16]. In these environments, sulphate concentrations usually significantly less than 0.5 mM allow methanogenic archaea to outcompete sulphate-reducing bacteria for their common substrates H_2 (ref. 17) and acetate[18], leading to enhanced methane generation. Increased levels of methane reaching the oxycline will, in turn, promote enhanced growth of aerobic methanotrophic bacteria. The high 3β-MHI in the BCF might therefore indirectly indicate sulphate concentrations considerably below modern marine levels (28 mM). This interpretation corroborates sulphide isotopic evidence from the overlying Reward Dolomite, the syndepositional shallow-water facies of the BCF. Reward shales contain pyrite with $\delta^{34}S$ averaging about +21.8 ± 1.8‰, which is consistent with pyrite precipitation from contemporaneous sea water strongly depleted in sulphate[5]. Isotope data from carbonate-associated sulphate from Australia and North America indicate that marine sulphate concentrations were below 0.5–2.5 mM between 1.7 and 1.3 Gyr ago[7], and $\delta^{34}S$ data on diagenetic pyrites from basins around the world indicate that sulphate depletion might have been a global phenomenon in this time interval[6,19]. The results of this biomarker study offer independent evidence that sulphate levels in the McArthur basin were significantly lower than in the present ocean.

In contrast to 3β-methylhopanes, the concentration of cyanobacterial 2α-methylhopanes is extremely low (C_{31}-2α-MHI < 1%; Fig. 1b). This attribute distinguishes these ancient bitumens from

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other Proterozoic carbonates, in which 2α-methylhopanes are almost always abundant[20]. However, not all cyanobacteria biosynthesize 2-methylhopanoids and so the paucity of these biomarkers does not exclude cyanobacteria as an important component of the ecosystem. On the contrary, cyanobacteria are the most likely source for the abundant β-carotane in the BCF (Fig. 1a) because potential precursor pigments are rare in phototrophic sulphur bacteria (PSB), and eukaryotic algae were not an important component of the ecosystem (see below). Cyanobacteria, together with green non-sulphur bacteria (Chloroflexaceae), are also the most likely source for γ-carotane in the BCF[21].

In strong contrast to most younger marine environments, biomarker abundances indicate that eukaryotic organisms might have had only an insignificant function in the offshore environment of the

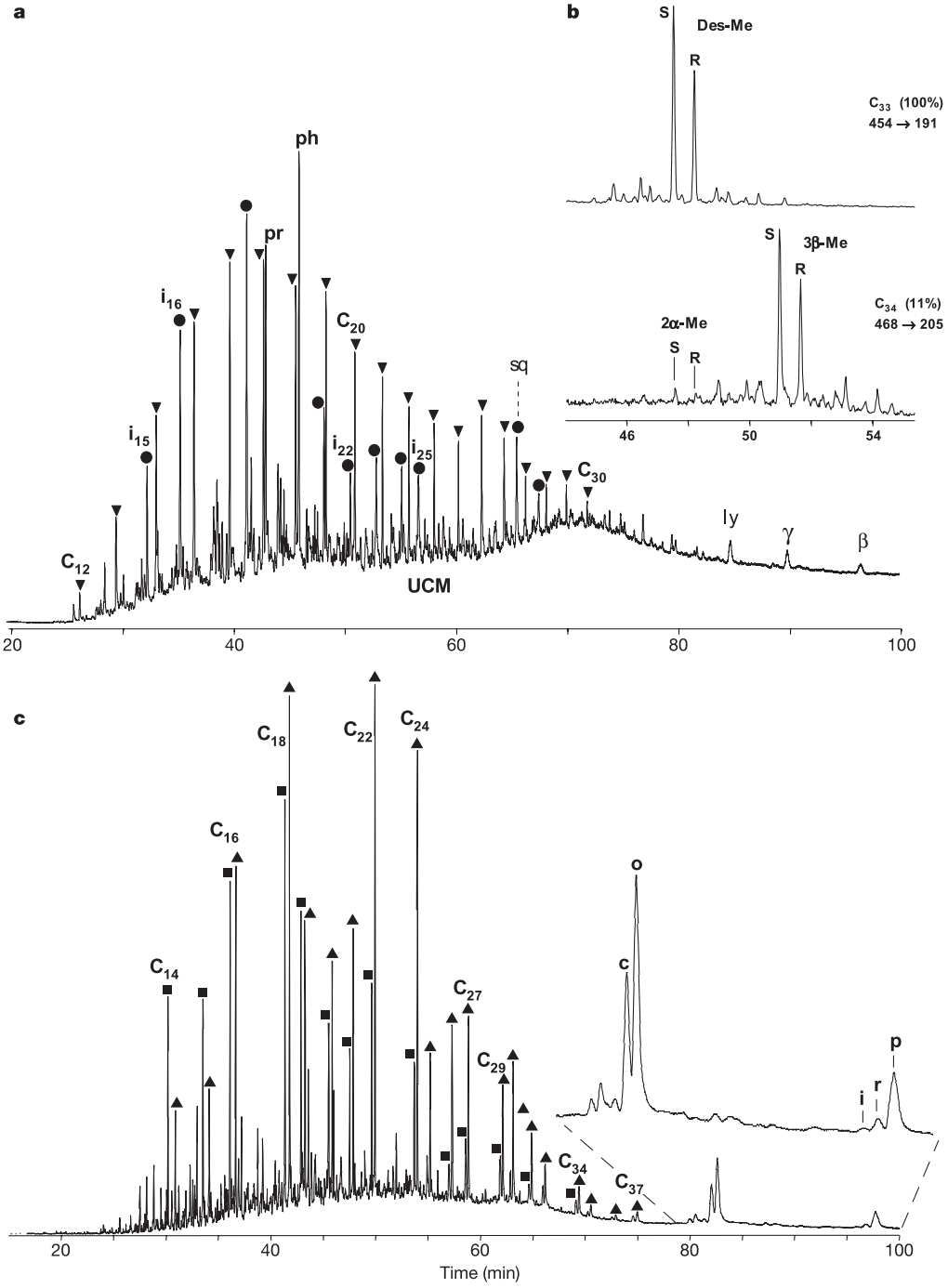

Figure 1 | **Biomarker distribution of the BCF. a**, Total ion recording of the saturated hydrocarbon fraction of sample B03065 (GR-7, 41.80 m). UCM, unresolved complex mixture; triangles, n-alkanes (C_x) with carbon number *x*; circles, acyclic isoprenoids (i_x) with carbon number *x*; sq, squalane; ly, lycopane; γ, tentatively identified γ-carotane; β, β-carotane. **b**, Multiple reaction monitoring of hopane hydrocarbons in sample B03071 (GR-7, 126.40 m). Chromatograms are identified by carbon numbers, the relative height (abundance) of the most intense peak in the trace, and the reaction transition from metastable molecular ions fragmenting to daughter ions at mass to charge ratio $m/z = 191$ for desmethylhopanes and $m/z = 205$ for methylhopanes. Top, 22(*S*) and 22(*R*) αβ-trishomohopane; bottom, their 2α- and 3β-methylated analogues. **c**, Selected ion recording at $m/z = 134.1$ of the aromatic fraction of sample B03066 (GR-7, 38.70 m). Squares, 2,3,6-trimethylarylisoprenoids; triangles, 2,3,4-trimethylarylisoprenoids; c, chlorobactane; o, okenane (see Fig. 3); i, isorenieratane; r, renieratane; p, renierapurpurane. Structures are shown in Supplementary Information.

BCF. Despite the excellent thermal preservation of the host-rocks, C_{26} to C_{29} steranes, biomarkers diagnostic for eukaryotes (and abundant in almost all Phanerozoic bitumens) were close to, or below, the detection limit of 1 p.p.m. In contrast, triaromatic steroids are commonly present at levels of 60–130 p.p.m. However, their distribution is highly unusual (Fig. 2a) and unlike Phanerozoic steroid assemblages (Fig. 2b). The triaromatic steroids entirely lack side-chain alkylation and are predominantly methylated at C-4 (more than 90%), a pattern indicating a possible bacterial source. A plausible biological source of the dominant C-4 methylated steroids is type I methanotrophic bacteria[22,23], offering further support for the abundance of these organisms in the BCF. The scarcity of diagnostic eukaryotic steroids in the analysed sample range, despite the abundance of steroids with a typical bacterial distribution, is a phenomenon that has never been observed before in any sedimentary sequence of any age and reflects severely restricted activity of eukaryotic algae in open waters.

The dominant biomarkers (up to 1,000 p.p.m.) in the aromatic hydrocarbon fractions are C_{14} to C_{37} 2,3,6- and 2,3,4-trimethyl arylisoprenoids (Fig. 1c), the typical breakdown products of aromatic carotenoids[24]. In the thermally least altered bitumens, it was possible to detect the intact precursor carotenoids chlorobactane, okenane (Fig. 3), isorenieratane, renieratane and renierapurpurane (chemical structures of the most relevant biomarkers, and original references and additional information about biological sources of aromatic carotenoids, can be found in the Supplementary Information). In living organisms, carotenoids with the renieratane and renierapurpurane skeletons are only known from sponges, in which they might be derived from as yet unknown bacterial sponge symbionts[24]. There is therefore no reliable biological information for interpreting these biomarkers in the BCF. In contrast, the biological precursors of chlorobactane and isorenieratane, the phototrophic pigments (hydroxy-) chlorobactene and isorenieratene, are well documented[24]. In deep aquatic environments, such as the BCF, these carotenoids are derived from planktonic green and brown pigmented species of Chlorobiaceae, respectively. Chlorobiaceae are strictly anaerobic, obligate phototrophs. They require hydrogen sulphide or other reduced sulphur species as an electron donor in the presence of light. Therefore, in planktonic environments, Chlorobiaceae thrive exclusively where euxinic conditions rise into the photic zone of the water column. The only known natural product with the okenane skeleton is okenone (Fig. 3), a red pigment found exclusively in several genera of planktonic purple sulphur bacteria of the family Chromatiaceae[25]. Although taxonomically unrelated, Chromatiaceae have physiological requirements similar to those of the Chlorobiaceae. Their preferred physiology is phototrophic oxidation of reduced sulphur. Thus, in planktonic environments, they thrive in a thin layer directly below the anoxic–oxic interface within the photic zone.

In modern environments, significant growth of Chromatiaceae occurs only at water depths of up to 20 m but usually much less[25]. Thus, okenone extracted from recent aquatic sediments most probably indicates an oxycline that ascended (temporarily) to a water depth of ~20 m or shallower. We consider this interpretation also as the most reasonable depth distribution for the source organisms of fossil okenane, assuming that the light requirements of planktonic Chromatiaceae have been stable through time and that planktonic activity and pigment densities in the overlying water column were comparable to those in modern stratified water systems. Similar depth considerations apply to the pigments of Chlorobiaceae. Green pigmented species commonly grow in a thin zone directly below purple sulphur bacteria and are reported at water depths up to ~13 m, whereas the brown pigmented species are adapted to very low light levels[25]. Brown pigmented green sulphur bacteria are regularly observed at water depths up to 18 m and, in the extreme case of the Black Sea, have been found at 80 m (ref. 26). In modern oceans, an upper mixed layer of less than 20 m is relatively common in warm coastal and even open marine regions[27]. Marine regions with an

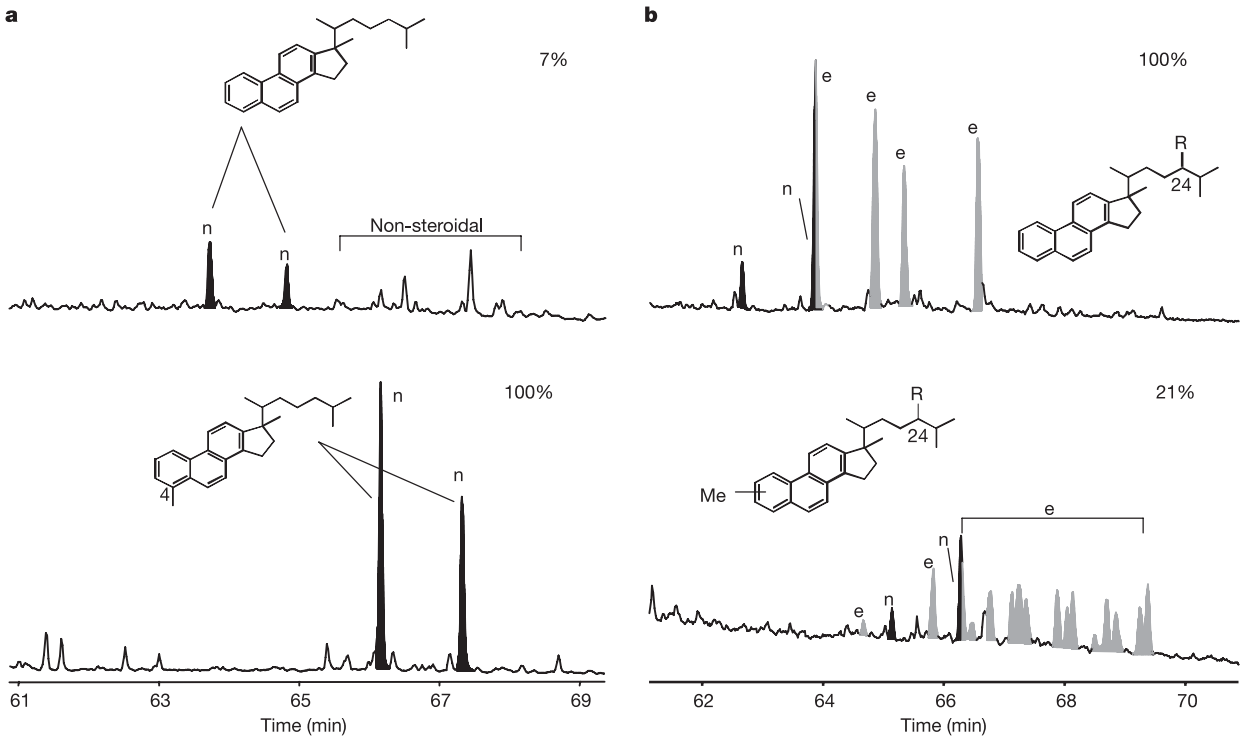

Figure 2 | Selected-ion-recording chromatograms of triaromatic steroids (TA; black and grey signals). Upper traces, $m/z = 231$; lower traces, $m/z = 245$. **a**, A representative sample of the Barney Creek Formation (B03063, GR-7, 180.00 m). **b**, A Phanerozoic petroleum mixture (AGSO Standard). Unmarked signals are non-steroidal. e (grey signals), diagnostic eukaryotic TA with alkylation at C-24 (R = methyl or ethyl); n (black signals), TA without side-chain alkylation (R = H). Percentages give the relative height (abundance) of the most intense peak in each trace.

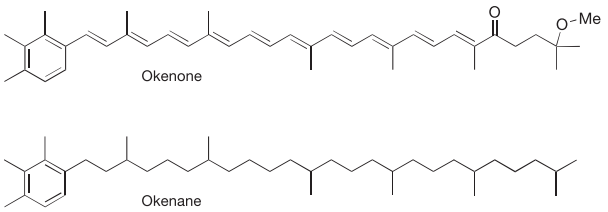

Figure 3 | Chemical structures of the carotenoid pigment okenone and its hydrocarbon fossil equivalent okenane.

upper mixed layer of less than 20 m might also have been widespread in the generally warm mid-Proterozoic, supplying ample marine habitat for anoxygenic phototrophs.

Until now, the oldest known chlorobactane was that reported from the Upper Jurassic[28], and we recently detected isorenieratane in the mid-Cambrian Currant Bush Limestone of the Georgina Basin, Australia. Thus, the current findings extend the fossil record of these biomarkers back in time by ~1.5 and ~1.1 Gyr, respectively. Although okenone is found in Recent sediments deposited under euxinic conditions[29], the equivalent fossil hydrocarbon okenane has not been reported from sedimentary rocks. It represents the only hydrocarbon biomarker, and in fact the only known fossil indicator, for Chromatiaceae. If okenane and other aromatic carotenoids in the BCF are correctly interpreted as molecular remains of PSB, then the McArthur Basin was intensely stratified and euxinic. The transition from oxic to anoxic conditions occurred just metres to a few tens of metres below the water surface. A shallow mixed layer with low oxygen concentrations and an episodic turbulent influx of H_2S might also explain the suppression of eukaryotic algae, poisoned under these conditions. During periods of widespread euxinia in the oxygen minimum zone, algae would also have suffered nitrogen stress and would have competed poorly against cyanobacteria[30]. The paucity of diagnostic eukaryotic biomarkers over the analysed range of the basin and over more than 800-m stratigraphic thickness in the Glyde River region indicates that the inferred conditions might have been maintained for extended periods.

Sedimentary successions demonstrating where and how the McArthur Basin was connected to the ocean are not preserved. However, accruing evidence indicates that the anoxic waters of the BCF might have been the extension of an oxygen-deficient ocean adjacent to the north Australian craton. On the eastern periphery of the craton, sulphur isotopes and iron speciation data from successions predating and postdating the BCF and with ages spanning ~1.73–1.49 Gyr indicate that deep-water anoxia and sulphate depletion were a common regional phenomenon[5,6]. Furthermore, molybdenum isotopes from the same 1.73-Gyr-old and 1.49-Gyr-old sedimentary sequences indicate that, globally, anoxic and dysoxic waters might have covered more of the ocean floor than they do today[8]. The rising evidence for widespread anoxia in mid-Proterozoic marine basins indicates that this was the prime age for planktonic PSB. In the preceding Archaean and early Palaeoproterozoic the supply of reduced sulphur was limited by an excess of dissolved ferrous iron in the oceans, causing rapid precipitation of iron sulphides. With the disappearance of banded iron formations, the availability of sulphide must have increased markedly[4], supporting blooms of PSB in the McArthur Basin and other marine regions where euxinic waters penetrated the photic zone.

The new biomarker results confirm hypotheses about late Palaeoproterozoic environments and life[30]. They record a euxinic marine ecosystem with comparatively low sulphate concentrations, high activity of methanotrophic bacteria, blooms of PSB and a paucity of eukaryotic algae in nutrient starved offshore habitats. The oxidation state of surface sea water seems to have risen again beginning at ~1.25 Gyr, perhaps in association with late Mesoproterozoic (Grenville) orogenesis, but oxygen levels and sulphate concentrations probably only reached more modern values later in the Neoproterozoic[30]. Further research on biomarkers detected in Proterozoic basins should help to clarify when widespread euxinic conditions retreated and eukaryotic algae expanded into a more nutrient-rich and oxygenated marine habitat.

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1. Holland, H. D. in *Treatise on Geochemistry* Vol. 6 *(The Oceans and Marine Geochemistry* (ed. Elderfield, H.) 583–625 (Elsevier/Pergamon, Oxford, 2004).
2. Farquhar, J., Bao, H. & Thiemens, M. Atmospheric influence of Earth's earliest sulfur cycle. *Science* **289,** 756–758 (2000).
3. Bekker, A. *et al.* Dating the rise of atmospheric oxygen. *Nature* **427,** 117–120 (2004).
4. Canfield, D. E. A new model for Proterozoic ocean chemistry. *Nature* **396,** 450–453 (1998).
5. Shen, Y., Canfield, D. E. & Knoll, A. H. Middle Proterozoic ocean chemistry: evidence from the McArthur Basin, northern Australia. *Am. J. Sci.* **302,** 81–109 (2002).
6. Shen, Y., Knoll, A. H. & Walter, M. R. Evidence for low sulphate and anoxia in a mid-Proterozoic marine basin. *Nature* **423,** 632–635 (2003).
7. Kah, L. C., Lyons, T. W. & Frank, T. D. Low marine sulphate and protracted oxygenation of the Proterozoic biosphere. *Nature* **431,** 834–838 (2004).
8. Arnold, G. L., Anbar, A. D., Barling, J. & Lyons, T. W. Molybdenum isotope evidence for widespread anoxia in mid-Proterozoic oceans. *Science* **304,** 87–90 (2004).
9. Page, R. W. & Sweet, I. P. Geochronology of basin phases in the western Mt Isa Inlier, and correlation with the McArthur Basin. *Aust. J. Earth Sci.* **45,** 219–232 (1998).
10. Bull, S. W. Sedimentology of the Palaeoproterozoic Barney Creek Formation in DDH BMR McArthur 2, southern McArthur Basin, Northern Territory. *Aust. J. Earth Sci.* **45,** 21–31 (1998).
11. Jackson, M. J., Southgate, P. N., Winefield, P. R., Barnett, K. & Zeilinger, I. Revised sub-devision and regional correlation of the McArthur Basin succession based on NABRE's 1995–8 sequence stratigraphic studies (Australian Geological Survey Organization, record 2000/03, Canberra, 2000).
12. Veizer, J., Plumb, K. A., Clayton, R. N., Hinton, R. W. & Grotzinger, J. P. Geochemistry of Precambrian carbonates: V. Late Paleoproterozoic seawater. *Geochim. Cosmochim. Acta* **56,** 2487–2501 (1992).
13. Crick, I. H., Boreham, C. J., Cook, A. C. & Powell, T. G. Petroleum geology and geochemistry of Middle Proterozoic McArthur Basin, northern Australia. II: Assessment of source rock potential. *AAPG Bull* **72,** 1495–1514 (1988).
14. Summons, R. E., Powell, T. G. & Boreham, C. J. Petroleum geology and geochemistry of the Middle Proterozoic McArthur Basin, northern Australia: III. Composition of extractable hydrocarbons. *Geochim. Cosmochim. Acta* **52,** 1747–1763 (1988).
15. Summons, R. E. & Jahnke, L. L. in *Biological Markers in Sediments and Petroleum* (eds Moldowan, J. M., Albrecht, P. & Philp, R. P.) 182–200 (Prentice Hall, Englewood Cliffs, New Jersey, 1992).
16. Collister, J. W., Summons, R. E., Lichtfouse, E. & Hayes, J. M. An isotopic biogeochemical study of the Green River oil shale. *Org. Geochem.* **19,** 265–276 (1992).
17. Hoehler, T. M., Alperin, M. J., Albert, D. B. & Martens, C. S. Thermodynamic control on hydrogen concentrations in anoxic sediments. *Geochim. Cosmochim. Acta* **62,** 1745–1756 (1998).
18. Scholten, J. C. M. *et al.* Effect of sulfate and nitrate on acetate conversion by anaerobic microorganisms in a freshwater sediment. *FEMS Microbiol. Ecol.* **42,** 375–385 (2002).
19. Hayes, J. M., Lambert, I. B. & Strauss, H. in *The Proterozoic Biosphere: A Multidisciplinary Study* (eds Schopf, J. W. & Klein, C.) 129–134 (Cambridge Univ. Press, New York, 1992).
20. Summons, R. E., Jahnke, L. L., Hope, J. M. & Logan, G. A. 2-Methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. *Nature* **400,** 554–557 (1999).
21. Palmisano, A. C., Cronin, S. E. & Des Marais, D. J. Analysis of lipophilic pigments from a phototrophic microbial mat community by high performance liquid chromatography. *J. Microbiol. Methods* **8,** 209–217 (1988).
22. Volkman, J. K. Sterols in microorganisms. *Appl. Microbiol. Biotechnol.* **60,** 496–506 (2003).
23. Bird, C. W. *et al.* Steroids and squalene in *Methylococcus capsulatus* grown on methane. *Nature* **230,** 473–474 (1971).
24. Brocks, J. J. & Summons, R. E. in *Treatise on Geochemistry* Vol. 8 *(Biogeochemistry)* (ed. Schlesinger, W. H.) 63–115 (Elsevier, Oxford, 2004).
25. Van Gemerden, H. & Mas, J. in *Anoxygenic Photosynthetic Bacteria* (eds Blankenship, R. E., Madigan, M. T. & Bauer, C. E.) 49–85 (Kluwer Academic, Dordrecht, 1995).
26. Repeta, D. J., Simpson, D. J., Jørgensen, B. B. & Jannasch, H. W. Evidence for the existence of anoxygenic photosynthesis from the distribution of bacteriochlorophylls in the Black Sea. *Nature* **342,** 69–72 (1989).
27. Kara, A. B., Rochford, P. A. & Hurlburt, H. E. Mixed layer depth variability over

the global ocean. *J. Geophys. Res.* **108**(C3), 3079 (2003) (doi:10.1029/2000JC000736).
28. van Kaam-Peters, H. M. E. & Sinninghe Damsté, J. S. Characterization of an extremely organic sulphur-rich, 150 Ma old carbonaceous rock: palaeoenvironmental implications. *Org. Geochem.* **27,** 371–397 (1997).
29. Schaeffer, P., Adam, P., Wehrung, P. & Albrecht, P. Novel aromatic carotenoid derivatives from sulfur photosynthetic bacteria in sediments. *Tetrahedr. Lett.* **38,** 8413–8416 (1997).
30. Anbar, A. D. & Knoll, A. H. Proterozoic ocean chemistry and evolution: a bioinorganic bridge? *Science* **297,** 1137–1142 (2002).

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