

## LETTERS

# Increased terrestrial methane cycling at the Palaeocene–Eocene thermal maximum

Richard D. Pancost<sup>1</sup>, David S. Steart<sup>2</sup>, Luke Handley<sup>1</sup>, Margaret E. Collinson<sup>2</sup>, Jerry J. Hooker<sup>3</sup>, Andrew C. Scott<sup>2</sup>, Nathalie V. Grassineau<sup>2</sup> & Ian J. Glasspool<sup>4</sup>

The Palaeocene–Eocene thermal maximum (PETM), a period of intense, global warming about 55 million years ago<sup>1</sup>, has been attributed to a rapid rise in greenhouse gas levels, with dissociation of methane hydrates being the most commonly invoked explanation<sup>2</sup>. It has been suggested previously that high-latitude methane emissions from terrestrial environments could have enhanced the warming effect<sup>3,4</sup>, but direct evidence for an increased methane flux from wetlands is lacking. The Cobham Lignite, a recently characterized expanded lacustrine/mire deposit in England, spans the onset of the PETM<sup>5</sup> and therefore provides an opportunity to examine the biogeochemical response of wetland-type ecosystems at that time. Here we report the occurrence of hopanoids, biomarkers derived from bacteria, in the mire sediments from Cobham. We measure a decrease in the carbon isotope values of the hopanoids at the onset of the PETM interval, which suggests an increase in the methanotroph population. We propose that this reflects an increase in methane production potentially driven by changes to a warmer<sup>1,6</sup> and wetter climate<sup>7,8</sup>. Our data suggest that the release of methane from the terrestrial biosphere increased and possibly acted as a positive feedback mechanism to global warming.

Although the PETM is not a direct analogue for future global warming because it occurs during a time when global temperatures were significantly higher than now<sup>9</sup>, it still allows the investigation of rapid warming comparable to that occurring today. A significant challenge in the investigation of terrestrial methane cycling at the PETM is the lack of appropriate sedimentary sequences. However, we have recently reported the identification of the PETM interval in lake and mire sediments from Cobham, southeast England<sup>5</sup>. The section, represented primarily by lignite through the PETM interval, provides a unique opportunity to study wetland microbial processes in a stratigraphically expanded and relatively complete continental section. The PETM is stratigraphically constrained (Methods) and characterized by a negative carbon isotope excursion (CIE) recorded by plant organic matter<sup>5</sup> (Fig. 1). The pronounced CIE is one of the defining characteristics of the PETM<sup>6,9</sup> and is typically attributed to the catastrophic release of <sup>13</sup>C-depleted methane from marine gas hydrates.

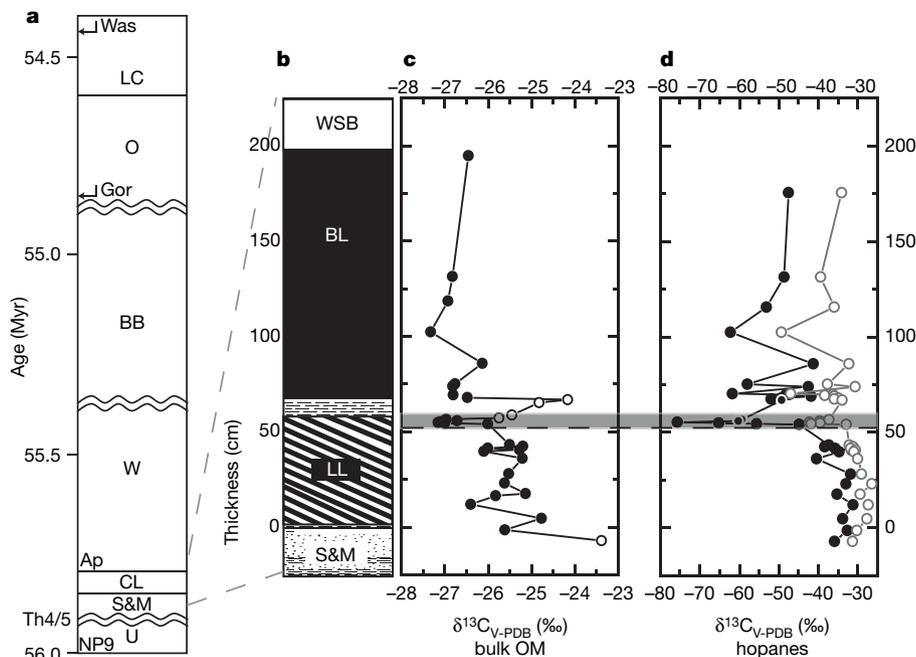
Critically, at Cobham, the 2-m sequence between the top of the Upnor Formation and the base of the WSB (Woolwich Shell Beds) is entirely freshwater. The black slickensided clay at the base of the sand and mud unit contains leech cocoons, termite coprolites, pollen and spores, but lacks dinoflagellates. The overlying sand (sand and mud unit) contains continental and freshwater pollen and spores and lacks the *Ophiomorpha* burrows indicative of saline conditions that are present in the underlying Upnor Formation. The Cobham Lignite

Bed (lignites and included thin clay bands) contains an abundance of continental and freshwater aquatic pollen and spores and algal cysts, and lacks dinoflagellates<sup>5,10</sup>; it is in this bed, with organic carbon contents ranging from 15 to 36 wt%, that the negative CIE has been recorded<sup>5</sup> (Fig. 1). A similar sequence with a freshwater lignite (although a thinner one than at Cobham) followed by brackish shelly clays, has been described from the north French coast at Varengeville; like Cobham, the CIE is recorded in the lignitic beds<sup>11</sup>, testifying to the extensive nature of the low-lying area of the southwest North Sea Basin at the time. Further to the east, in north Belgium, the Doel and Kallo boreholes, which are down dip from Kent in the North Sea Basin, show that the CIE occurs in a mixed freshwater and brackish sequence<sup>12</sup>. Thus, the nearest occurrence of contemporaneous saline conditions is perhaps hundreds of kilometres from the Cobham Lignite studied here. Such a setting provides an ideal, perhaps even unique, opportunity to examine the biogeochemical response of a continental lacustrine/mire system to increased global temperature at the PETM.

Examination of microbially mediated biogeochemical processes in ancient sediments can be challenging, but biomarkers (organic compounds that can be structurally related to biological precursors) have proved to be useful in studying the ancient microbiology of, for example, the Permo-Triassic boundary<sup>13</sup>. In the Cobham Lignite, the hydrocarbon distribution is dominated by *n*-alkanes with an odd-over-even carbon number predominance indicative of a higher-plant origin<sup>14</sup> and exceptional abundances of hopanes and hopenes (Fig. 2) derived from bacteria<sup>15</sup>. Triterpenes and sterenes of inferred higher-plant origin are also present but in subordinate abundances. In the polar fractions, which are discussed here only briefly, *n*-alkanols and sterols, also of inferred higher-plant origin, are predominant.

The hopanes are dominated by the C<sub>29</sub>, C<sub>30</sub> and C<sub>31</sub> homologues (21-norhopane, hopane and homohopane, respectively), with the 17 $\alpha$ ,21 $\beta$ (H), 17 $\beta$ ,21 $\beta$ (H) and 17 $\beta$ ,21 $\alpha$ (H) isomers all present. The presence of abundant hopanes with the biological  $\beta\beta$  configuration and the lack of hopanes with 22S stereochemistry, combined with high abundances of hopenes, indicate that the Cobham Lignite is relatively immature with regard to petroleum generation, which is consistent with vitrinite reflectance values ( $R_o = 0.39 \pm 0.1$  (mean  $\pm$  s.d.))<sup>10</sup>. Traditionally, hopanes and their precursor hopanoids have been attributed to aerobic bacteria, but recent work has revealed an anaerobic origin for hopanoids as well<sup>16</sup>; consequently, the interpretation of hopanoid distributions and depth profiles is complex. In the Cobham Lignite, further complexity is imposed by marked variations in hopanoid distributions and stereochemistry, the latter possibly being due to changing environmental conditions during sediment deposition<sup>17</sup>.

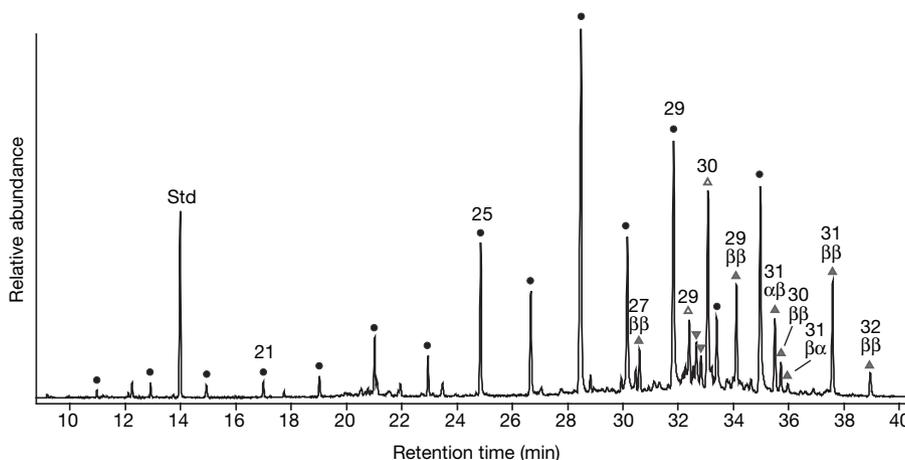
<sup>1</sup>Organic Geochemistry Unit, Bristol Biogeochemistry Research Centre, School of Chemistry, University of Bristol, Cantock's Close, Bristol BS8 1TS, UK. <sup>2</sup>Department of Geology, Royal Holloway University of London, Egham, Surrey TW20 0EX, UK. <sup>3</sup>Palaeontology Department, Natural History Museum, Cromwell Road, London SW7 5BD, UK. <sup>4</sup>Department of Geology, The Field Museum, 1400 S. Lake Shore Drive, Chicago, Illinois 60605, USA.



**Figure 1 | Depth profile through the Cobham Lignite.** **a**, Position of the profile in the temporally calibrated latest Palaeocene and Early Eocene East London Basin sequence. U, Upnor Formation; S&M, sand and mud unit; CL, Cobham Lignite; W, Woolwich Formation; BB, Blackheath Beds; O, Oldhaven Member; LC, London Clay Formation. Ap denotes *Apectodinium*, Gor and Was refer to dinocyst zones, and Th4/5 denotes a sequence boundary. For further details, see Methods. **b**, Lithologic log of the CL (BL, blocky lignite; LL, laminated lignite); WSB, lower Woolwich Shell Beds.

More specific biological source information can be obtained from the carbon isotopic composition of the hopanes (Fig. 1d). In the lower part of the lignite and the underlying sand and mud unit,  $\delta^{13}\text{C}$  values of the  $\text{C}_{29}$  hopane ( $17\beta,21\beta(\text{H})$  isomer) are about  $-36\text{‰}$  and values of the  $\text{C}_{31}$  hopane ( $17\beta,21\beta(\text{H})$  isomer) range from  $-31$  to  $-30.5\text{‰}$ . These values are slightly depleted relative to co-occurring bulk organic matter ( $-26$  to  $-24.5\text{‰}$ ) and higher-plant *n*-alkanes ( $-30.5$  to  $-29.5\text{‰}$ ). The carbon isotopic compositions of hopanes in some recent wetlands are typically enriched in  $^{13}\text{C}$  relative to these values ( $-26$  to  $-22\text{‰}$ ) and relative to co-occurring higher-plant biomarkers<sup>17</sup>; this has been attributed to a heterotrophic ecology in which the source bacteria were consuming  $^{13}\text{C}$ -enriched carbohydrates. In the Cobham Lignite, the lower  $\delta^{13}\text{C}$  values suggest that the hopanes derive from a mixture of heterotrophic bacteria that

consumed higher-plant-derived organic matter and methanotrophs that were significantly depleted in  $^{13}\text{C}$  (there is no biomarker evidence for cyanobacteria). At the base of the PETM as indicated by a shift in bulk organic  $\delta^{13}\text{C}$  values from  $-26$  to  $-27.6\text{‰}$  (Fig. 1c), hopane  $\delta^{13}\text{C}$  values decrease markedly to  $-42\text{‰}$  and  $-76\text{‰}$  for the  $\text{C}_{31}$  and  $\text{C}_{29}$  hopanes, respectively. In fact, all hopane and hopene  $\delta^{13}\text{C}$  values decrease significantly through this depth interval, but accurate values could be determined only for the components shown in Fig. 1 because of co-elution or peak size. Such a preponderance of hopanes with very low  $\delta^{13}\text{C}$  values is unusual in the geological record, with reported occurrences limited to only a few methanogenic lacustrine settings<sup>18–20</sup> and cold-seep carbonate deposits. We know of no other modern or ancient mire deposit characterized by such  $^{13}\text{C}$ -depleted hopanoids.



**Figure 2 | Gas chromatogram of a typical Cobham Lignite apolar (hydrocarbon) fraction.** The sample shown is from a depth of 55.3 cm, coinciding with the most negative hopanoid  $\delta^{13}\text{C}$  values). Filled circles,

*n*-alkanes; filled triangles, hopanes with stereochemistry shown above; open triangles, hopenes; inverted triangles,  $\text{C}_{29}$  sterenes. In all cases, numbers indicate the number of carbon atoms in the molecule. Std, standard.

The shift in hopane  $\delta^{13}\text{C}$  values could be due to a decrease in heterotrophic bacterial biomass or an increase in the total methanotroph biomass. In the Cobham Lignite, hopane concentrations vary significantly, but neither their absolute abundances (relative to total lignite) nor their abundances relative to higher-plant *n*-alkanes covary with their carbon isotopic compositions. In fact, abundances stay relatively constant during the most marked carbon isotopic shift, suggesting that the hopane CIE is not due solely to decreased inputs from heterotrophic bacteria but is instead a combination of decreased heterotrophic bacterial inputs coinciding with increased methanotroph inputs. As with all proxies, caution must be used when interpreting biomarker trends; for example, the decrease in  $\delta^{13}\text{C}$  values could also indicate a change in the depositional environment that favours the preservation of methanotroph-derived hopanoids. Such an explanation seems unlikely because the shift in  $\delta^{13}\text{C}$  values occurs before any major lithologic change (Fig. 1), and no  $^{13}\text{C}$ -depleted compounds were observed before the PETM. The unusual dominance of  $^{13}\text{C}$ -depleted hopanoids at the PETM is unlikely to be an artefact of a change in preservation that is unaccompanied by evidence for a change in depositional environment. Thus, we interpret the shift in hopane  $\delta^{13}\text{C}$  values as evidence for increased methanotroph biomass and, possibly, decreased heterotroph biomass. Because methanotrophy generally tracks methane concentrations<sup>21</sup>, the increase in methanotroph hopanes was almost certainly a response to increased methane flux from deeper sediments; in fact, increased methanogenic organic matter degradation would also be consistent with the inferred decrease in hopane contributions from heterotrophic bacteria.

Although the Cobham Lignite is a unique deposit and comparable records have not yet been explored, it provides a useful analogue of how such settings might respond to the warming and associated climatic changes that occurred at the PETM. Higher temperatures increase the rate of methanogenesis in modern settings<sup>22</sup>; consequently, warming could have exerted a direct control on methane release from the Cobham setting and similar environments. Alternatively or additionally, wetter conditions are also associated with elevated methane fluxes from wetlands, and there is evidence that the climate local to the Cobham Lignite became wetter at the PETM. The lower laminated lignite was deposited in a shallow lacustrine/mire setting, a situation that persisted through the base of the CIE and the corresponding shift in hopane  $\delta^{13}\text{C}$  values. However, in the uppermost 2 cm of the laminated lignite, corresponding to the lowest hopane  $\delta^{13}\text{C}$  values (as denoted by the horizontal grey bar in Fig. 1), there is a marked decrease in charcoal content and an increase in clay-rich laminae, which together have been interpreted as evidence for increased precipitation and/or humidity over the surrounding area<sup>23</sup>. Deposition of the laminated lignite was followed conformably by a thin clay and then accumulation of the charcoal-poor blocky lignite. Because the change from laminated to blocky lignite lithology followed the negative shift in hopane  $\delta^{13}\text{C}$  values, it seems to be an unlikely mechanism for increased rates of methanogenesis. Instead, changes in the hydrological regime (and possibly warming) could have brought about increased methanogenesis in the Cobham depositional setting. As there have been several studies suggesting elevated humidity<sup>7</sup> or precipitation at the PETM<sup>8,24,25</sup>, similar increases in methane cycling could have occurred at other locations. Potentially, such increased rates of methanogenesis and methanotrophy in continental settings could have contributed to changes in the global carbon cycle at the PETM.

Recent work, including new records of carbonate compensation depth shoaling<sup>26</sup> and higher-plant biomarker  $\delta^{13}\text{C}$  values in Arctic sediments<sup>27</sup>, has been used to argue for a greater input of carbon than that suggested by the methane hydrate model<sup>2</sup>. Enhanced terrestrial methane inputs from Cobham and similar settings could have served as an additional source of carbon, but we consider this unlikely because increased methanogenesis would probably occur at the expense of  $\text{CO}_2$  emissions, and the net carbon input into the

atmosphere would remain constant. However, wetlands are the dominant natural source of atmospheric methane on the Earth today<sup>22</sup>, and increased continental methane emissions could have been important. Continental methane sources are thought to be important components of glacial–interglacial changes in atmospheric methane concentrations, with enhanced methane production in tropical wetlands possibly accounting for 70% of the increase in atmospheric methane since the last deglaciation<sup>28</sup>. Although this is equivalent to only about 250 Gt of methane, the magnitude of methane release from wetlands could have been greater at the PETM if large quantities of organic carbon had been sequestered in peatlands during the Palaeocene<sup>29</sup>; indeed, previous workers have suggested that increased methane fluxes from wetlands could have contributed to warming at the PETM<sup>3,4,7</sup>. In particular, it was argued<sup>3</sup> that such an increased flux contributed to high-latitude warming by the formation of polar stratospheric clouds; given new data suggesting that sea surface temperatures in the Arctic Ocean were higher than models have predicted<sup>30</sup>, the role of atmospheric methane as a positive feedback on climate change could have been significant.

Of course, caution is necessary when extrapolating our observations in the Cobham Lignite to a global scale: many previous workers have commented on the complex controls governing methane flux from wetlands and similar environments, with particular emphasis placed on not just changes in precipitation and temperature but also vegetation and the antecedent hydrological conditions. However, our work, especially when combined with globally diverse evidence for increasing precipitation or continental runoff at the PETM<sup>8,25</sup>, suggests that terrestrial environments could produce more methane in response to global warming both at the PETM and in the future.

## METHODS SUMMARY

In general, all of the East London Basin sequence from the latest Palaeocene to the Early Eocene was exposed at Cobham (Fig. 1a) with the sand and mud unit (S&M), Cobham Lignite (CL) and lower Woolwich Shell Beds (W) all shown in expanded view in Fig. 1b. Analytical methods employed in this study focused on the determination of both bulk and specific biomarker carbon isotopic compositions (expressed in  $\delta^{13}\text{C}$  notation relative to Vienna Pee Dee Belemnite (V-PDB) carbonate standard). Carbon isotopic measurements on bulk samples were obtained with a high-resolution He-continuous flow mass spectrometry technique (EA-IRMS), and the reproducibility on the standards is better than  $\pm 0.1\%$ . For biomarker analyses, samples were gently washed with methanol, powdered and extracted by sonication; resultant lipid extracts were separated into three fractions on a column packed with (activated) alumina. GC-MS was performed with a Thermoquest Finnigan Trace GC interfaced to a Thermoquest Finnigan Trace MS, and GC-IRMS was performed with a ThermoFinnigan DeltaS. For both, a fused silica capillary column (50 m  $\times$  0.32 mm) coated with CP-Sil-5 (film thickness 0.12  $\mu\text{m}$ ) was used with the following temperature programme: 40  $^\circ\text{C}$  to 140  $^\circ\text{C}$  at 20  $^\circ\text{C min}^{-1}$ , then to 300  $^\circ\text{C}$  at 4  $^\circ\text{C min}^{-1}$ , maintained at 300  $^\circ\text{C}$  for 22 min. For compound-specific carbon isotopic analyses, the reproducibility on the standards is better than  $\pm 0.3\%$ .

**Full Methods** and any associated references are available in the online version of the paper at [www.nature.com/nature](http://www.nature.com/nature).

**Received 12 December 2006; accepted 8 June 2007.**

- Zachos, J. C. *et al.* A transient rise in tropical sea surface temperature during the Paleocene–Eocene Thermal Maximum. *Science* **302**, 1551–1554 (2003).
- Dickens, G. R. Rethinking the global carbon cycle with a large, dynamic and microbially mediated gas hydrate capacitor. *Earth Planet. Sci. Lett.* **213**, 169–183 (2003).
- Sloan, L. C., Walker, J. C. G., Moore, T. C., Rea, D. K. & Zachos, J. C. Possible methane-induced polar warming in the early Eocene. *Nature* **357**, 320–322 (1992).
- Sloan, L. C. & Pollard, D. Polar stratospheric clouds: A high latitude warming mechanism in an ancient greenhouse world. *Geophys. Res. Lett.* **25**, 3517–3520 (1998).
- Collinson, M. E., Hooker, J. J. & Gröcke, D. R. in *Causes and Consequences of Globally Warm Climates in the Early Paleogene* (eds Wing, S. L., Gingerich, P. D., Schmitz, B. & Thomas, B.) 333–349 (Geol. Soc. Am. Special Paper 369, Boulder, Colorado, 2003).

6. Kennett, J. P. & Stott, L. D. Abrupt deep-sea warming, palaeoceanographic changes and benthic extinctions at the end of the Palaeocene. *Nature* **353**, 225–229 (1991).
  7. Bowen, G. J., Beerling, D. J., Koch, P. L., Zachos, J. C. & Quattlebaum, T. A humid climate state during the Palaeocene/Eocene thermal maximum. *Nature* **432**, 495–499 (2004).
  8. Crouch, E. M. *et al.* The *Apectodinium* acme and terrestrial discharge during the Paleocene–Eocene thermal maximum: new palynological, geochemical and calcareous nannoplankton observations at Tawanui, New Zealand. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **194**, 387–403 (2003).
  9. Zachos, J., Pagani, M., Sloan, L., Thomas, E. & Billups, K. Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* **292**, 686–693 (2001).
  10. Collinson, M. E., Steart, D. C., Scott, A. C., Glasspool, I. J. & Hooker, J. J. Episodic fire, run-off and deposition at the Palaeocene–Eocene boundary. *J. Geol. Soc. Lond.* **164**, 87–97 (2007).
  11. Magioncalda, R. *et al.* L'excursion isotopique du carbone organique ( $\delta^{13}\text{C}$ ) dans les paléoenvironnements continentaux de l'intervalle Paléocène/Eocène de Varengeville (Haute-Normandie). *Bull. Soc. Geol. Fr.* **172**, 349–358 (2001).
  12. Steurbaut, E. *et al.* Palynology, palaeoenvironments, and organic carbon isotope evolution in lagoonal Paleocene–Eocene boundary settings in North Belgium. *GSA Spec. Pap.* **369**, 291–317 (2003).
  13. Xie, S. C., Pancost, R. D., Yin, H. F., Wang, H. M. & Evershed, R. P. Two episodes of microbial change coupled with Permo/Triassic faunal mass extinction. *Nature* **434**, 494–497 (2005).
  14. Eglinton, G. & Hamilton, R. J. Leaf epicuticular waxes. *Science* **156**, 1322–1334 (1967).
  15. Rohmer, M., Bouviernave, P. & Ourisson, G. Distribution of hopanoid triterpenes in prokaryotes. *J. Gen. Microbiol.* **130**, 1137–1150 (1984).
  16. Hartner, T., Straub, K. L. & Kannenberg, E. Occurrence of hopanoid lipids in anaerobic *Geobacter* species. *FEMS Microbiol. Lett.* **243**, 59–64 (2005).
  17. Pancost, R. D., Baas, M., van Geel, B. & Damste, J. S. S. Response of an ombrotrophic bog to a regional climate event revealed by macrofossil, molecular and carbon isotopic data. *Holocene* **13**, 921–932 (2003).
  18. 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).
  19. Ruble, T. E., Bakel, A. J. & Philp, R. P. Compound-specific isotopic variability in Uinta Basin native bitumens—paleoenvironmental implications. *Org. Geochem.* **21**, 661–671 (1994).
  20. Schouten, S. *et al.* Molecular organic tracers of biogeochemical processes in a saline meromictic lake (Ace Lake). *Geochim. Cosmochim. Acta* **65**, 1629–1640 (2001).
  21. Inubushi, K. *et al.* Factors influencing methane emission from peat soils: Comparison of tropical and temperate wetlands. *Nutrient Cycling Agroecosyst.* **71**, 93–99 (2005).
  22. Christensen, T. R. *et al.* Factors controlling large scale variations in methane emissions from wetlands. *Geophys. Res. Lett.* **30**, 1414, doi:10.1029/2002GL016848 (2003).
  23. Steart, D. C., Collinson, M. E., Scott, A. C., Glasspool, I. J. & Hooker, J. J. The Cobham Lignite Bed: the palaeobotany of two petrographically contrasting lignites from either side of the Paleocene–Eocene carbon isotope excursion. *Acta Palaeobot* **47** (1), 109–125 (2007).
  24. Schmitz, B. & Pujalte, V. Abrupt increase in seasonal extreme precipitation at the Paleocene–Eocene boundary. *Geology* **35**, 215–218 (2007).
  25. Gibson, T. G., Bybell, L. M. & Mason, D. B. Stratigraphic and climatic implications of clay mineral changes around the Paleocene/Eocene boundary of the northeastern US margin. *Sedim. Geol.* **134**, 65–92 (2000).
  26. Zachos, J. C. *et al.* Rapid acidification of the ocean during the Paleocene–Eocene thermal maximum. *Science* **308**, 1611–1615 (2005).
  27. Pagani, M. *et al.* Arctic hydrology during global warming at the Palaeocene/Eocene thermal maximum. *Nature* **442**, 671–675 (2006).
  28. Maslin, M. A. & Thomas, E. Balancing the deglacial global carbon budget: the hydrate factor. *Quat. Sci. Rev.* **22**, 1729–1736 (2003).
  29. Kurtz, A. C., Kump, L. R., Arthur, M. A., Zachos, J. C. & Paytan, A. Early Cenozoic decoupling of the global carbon and sulfur cycles. *Paleoceanography* **18**, doi:10.1029/2003PA000908 (2003).
  30. Sluijs, A. *et al.* Subtropical arctic ocean temperatures during the Palaeocene/Eocene thermal maximum. *Nature* **441**, 610–613 (2006).
- Acknowledgements** We thank I. D. Bull and R. Berstan of the Bristol Node of the NERC Life Sciences Mass Spectrometry Facility (LSMSF) for analytical support; Alfred McAlpine plc, AMEC and Channel Tunnel Rail Link for access to the Cobham Lignite Bed, and S. Rose for making arrangements; J. Skipper and S. Tracey for help with initial sample collection and field discussions; and L. Kump for critical comments and advice on the ideas proposed here. We acknowledge funding support for this research from the Leverhulme Trust, and studentship support for L.H. from the NERC.
- Author Contributions** R.D.P. and M.E.C. contributed equally to this paper. M.E.C., J.J.H. and A.C.S. initially characterized the Cobham Lignite, with I.J.G., and led the subsequent more detailed characterization of the sediments and fossils conducted by D.S.S. R.D.P. led the biomarker analyses at Bristol, with most of the preparation and analyses being conducted by D.S.S., with training and assistance from L.H. N.V.G. conducted bulk organic matter isotopic measurements. R.D.P. wrote the paper, and all authors discussed the results and commented on the manuscript.
- Author Information** Reprints and permissions information is available at [www.nature.com/reprints](http://www.nature.com/reprints). The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to R.D.P. (r.d.pancost@bristol.ac.uk).

## METHODS

**Stratigraphy.** Figure 1a shows a simplified East London Basin sequence from latest Palaeocene to Early Eocene calibrated to the latest geological timescale<sup>31</sup>. All of these units were exposed at Cobham. Figure 1a shows the positions of the sand and mud unit (S&M), Cobham Lignite (CL) and lower Woolwich Shell Beds (part of the Woolwich Formation, W), all of which are shown in expanded view in Fig. 1b. The Cobham Section (Fig. 1b) is underlain by the Upnor Formation (U), which is dated as latest Palaeocene by means of the occurrence of calcareous nannoplankton zone NP9 and magnetochron C25n in its lower part<sup>32</sup>. The Upnor Formation is separated from overlying units by a major unconformity marking the Th4/5 sequence boundary<sup>33</sup> (unconformities and missing strata are denoted in Fig. 1a by double undulating lines). The lower Woolwich Shell Beds, containing the *Apectodinium* acme (Ap), are overlain successively by the rest of the Woolwich Formation, the Blackheath Beds (BB), Oldhaven Member (O) and the London Clay Formation (LC). The last two are dated by means of the Early Eocene dinocyst zones Gor and Was<sup>33,34</sup>. See ref. 5 for a full discussion of the dating.

The presence of calcareous nannoplankton and *Ophiomorpha* burrows indicates a marine depositional environment for the Upnor Formation. The sand and mud unit, and both the laminated and blocky lignites and the clay bands of the Cobham Lignite, were deposited in a freshwater environment as explained in the text. Furthermore, the sequence boundary (Th4/5) between the Upnor Formation and the sand and mud unit is major, such that there is no brackish transition between the two. The sand and mud unit thus represents the initial filling of an eroded topography during base level rise, saline conditions not returning until the lower Woolwich Shell Beds.

**Analytical methods.** The Cobham Lignite and related sediments were collected and stored as described previously<sup>5,10</sup>. Carbon isotopic measurements on bulk samples were obtained with a high-resolution He-continuous flow mass spectrometry technique (EA-IRMS), by means of an elemental analyser EA-1500 (Fisons) coupled with an Optima GV-Micromass MS (described in ref. 35). The samples were cleaned in methanol in an ultrasonic bath for 10 min and then dried. The quantity analysed depended on the carbon content in the sample, with weights between 0.085 and 0.420 mg. Four standards were used to calibrate the results, two international standards (NBS 21 graphite and IAEA-CO9 barium carbonate), and two working minerals (a calcite and a graphite), covering a  $\delta^{13}\text{C}$  range from  $-47$  to  $+3\%$ . The reproducibility on the standards is better than  $\pm 0.1\%$ . The carbon isotopic composition is expressed also in conventional  $\delta^{13}\text{C}$  notation as per-thousand ( $\%$ ) variations relative to Vienna Pee Dee Belemnite (V-PDB) carbonate standard.

For biomarker analyses, samples were gently washed with methanol and powdered with a mortar and pestle. The powdered samples were extracted by sonication with a sequence of increasingly polar solvents (four times with dichloromethane (DCM), four times with DCM/methanol (1:1 v/v) and three times with methanol). The total lipid extracts were separated into three fractions by using a column packed with (activated) alumina by elution with hexane (apolar fraction), hexane/DCM (9:1 v/v; 3 ml) and DCM/methanol (1:2 v/v; 3 ml; polar fraction). GC-MS was performed with a Thermoquest Finnigan trace GC, equipped with an on-column injector and using helium as the carrier gas, interfaced to a Thermoquest Finnigan Trace MS. A fused silica capillary column (50 m  $\times$  0.32 mm) coated with CP-Sil-5 (film thickness 0.12  $\mu\text{m}$ ) was used. Samples were injected at 40 °C and the oven was programmed to increase to 140 °C at 20 °C min<sup>-1</sup> and then to 300 °C at 4 °C min<sup>-1</sup>, at which temperature it was held for 22 min. The mass spectrometer was operated with electron ionization at 70 eV and scanning a mass range of  $m/z$  50–700 using a cycle time of 1.7 scans s<sup>-1</sup>. The interface was set to 300 °C with the ion source at 240 °C. Carbon-isotopic ratios of individual compounds were determined by gas chromatography–isotope ratio mass spectrometry (GC-IRMS) with a ThermoFinnigan DeltaS and the same column and run conditions as described above.  $\delta^{13}\text{C}$  values relative to V-PDB were calculated by comparison with a calibrated CO<sub>2</sub> gas; the uncertainties, determined by using co-injected standards, are  $\pm 0.3\%$ .

31. Gradstein, F. M., Ogg, J. G. & Smith, A. G. (eds) *A Geologic Time Scale 2004* (Cambridge University Press, Cambridge, 2004).
32. Ellison, R. A., Ali, J. R., Hine, N. M. & Jolley, D. W. in *Correlation of the Early Paleogene in Northwest Europe* (eds Knox, R. W. O'B., Corfield, R. M. & Dunay, R. E.) 185–193 (Geol. Soc. Lond. Special Publication 101, 1996).
33. Bujak, J. P. & Brinkhuis, H. in *Late Paleocene–Early Eocene Climatic and Biotic Events in the Marine and Terrestrial Records* (eds Aubry, M.-P., Lucas, S. G. & Berggren, W. A.) 277–295 (Columbia University Press, New York, 1998).
34. Powell, A. J. in *A Stratigraphic Index of Dinoflagellate Cysts* (ed. Powell, A. J.) 155–252 (Br. Micropalaeontol. Soc. Publ. Series, Chapman & Hall, London, 1992).
35. Grassineau, N. V. High-precision EA-IRMS analysis of S and C isotopes in geological materials. *Appl. Geochem.* 21, 756–765 (2006).