Title
Temperature limits to deep subseafloor life in the Nankai Trough subduction zone

Journal
Science, 370(6521)

ISSN
0036-8075

Authors
Heuer, Verena B
Inagaki, Fumio
Morono, Yuki
et al.

Publication Date
2020-12-04

DOI
10.1126/science.abd7934

Peer reviewed

DEEP BIOSPHERE

Temperature limits to deep subseafloor life in the Nankai Trough subduction zone

Verena B. Heuer[1]*, Fumio Inagaki[2,3]*†, Yuki Morono[3]*, Yusuke Kubo[4]‡, Arthur J. Spivack[5], Bernhard Viehweger[1], Tina Treude[6], Felix Beulig[7]§, Florence Schubotz[1], Satoshi Tonai[8], Stephen A. Bowden[9], Margaret Cramm[10]¶, Susann Henkel[11], Takehiro Hirose[3], Kira Homola[5], Tatsuhiko Hoshino[3], Akira Ijiri[3], Hiroyuki Imachi[12], Nana Kamiya[13]#, Masanori Kaneko[14], Lorenzo Lagostina[15]**, Hayley Manners[16], Harry-Luke McClelland[17]††, Kyle Metcalfe[18], Natsumi Okutsu[19]†, Donald Pan[20]‡‡, Maija J. Raudsepp[21]§§, Justine Sauvage[5]¶¶, Man-Yin Tsang[22], David T. Wang[23]##, Emily Whitaker[24], Yuzuru Yamamoto[25]***, Kiho Yang[26]†††, Lena Maeda[4]†, Rishi R. Adhikari[1], Clemens Glombitza[27], Yohei Hamada[3], Jens Kallmeyer[28], Jenny Wendt[1], Lars Wörmer[1], Yasuhiro Yamada[2]†, Masataka Kinoshita[29], Kai-Uwe Hinrichs[1]‡‡‡

Microorganisms in marine subsurface sediments substantially contribute to global biomass. Sediments warmer than 40°C account for roughly half the marine sediment volume, but the processes mediated by microbial populations in these hard-to-access environments are poorly understood. We investigated microbial life in up to 1.2-kilometer-deep and up to 120°C hot sediments in the Nankai Trough subduction zone. Above 45°C, concentrations of vegetative cells drop two orders of magnitude and endospores become more than 6000 times more abundant than vegetative cells. Methane is biologically produced and oxidized until sediments reach 80° to 85°C. In 100° to 120°C sediments, isotopic evidence and increased cell concentrations demonstrate the activity of acetate-degrading hyperthermophiles. Above 45°C, populated zones alternate with zones up to 192 meters thick where microbes were undetectable.

Scientific ocean drilling has demonstrated the ubiquity of microbial life in deep subseafloor environments down to 2.5 km below seafloor (*1*–*3*). Because sediment temperature increases with burial depth, more than 50% of the global marine sediment volume is situated above 40°C (*4*). So far, the vast majority of subseafloor-life studies have targeted environments with in situ temperatures <30°C; the habitability of hotter sediments is largely unexplored. Microbes with growth temperatures up to 122°C have been isolated at hydrothermal vents (*5*), where the metabolism of these hyperthermophiles is fueled by high fluxes of oxidants and reductants (*6*). However, in deeply buried sediments, energy is limited, and with increasing depth and temperature, the slow-growing microbial communities struggle to meet the cellular maintenance energy requirement (*3*, *7*, *8*). Even in organic matter–rich petroleum reservoirs, microbial activity appears to cease at temperatures of ~80°C (*9*, *10*).

Aiming to fill the vast knowledge gaps regarding the response of microbial life to increasing temperature, we investigated up to 1.2-km-deep and up to 120°C hot sediments in the Nankai Trough off Cape Muroto, Japan (fig. S1). In this area, an up to 16-million-year-old, ~600-m-thick succession of hemipelagic mudstones and tuffs has been rapidly buried by an equally thick layer of trench deposits over the past ~0.4 million years (My) [(*11*); fig. S2]. Sediments concurrently heated by about 50°C and the onset of subduction formed a décollement separating the accreting and underthrusted domains (*11*, *12*). First indications for the presence of microbial life in ~800-m-deep, ~80° to 90°C warm sediments at a nearby drill site date back two decades (*11*, *12*). However, insufficient sensitivity in cell detection at that time compromised the habitability assessment of this environment (*11*, *12*). We designed Expedition 370 of the International Ocean Discovery Program (IODP) to achieve maximal sensitivity in life detection together with accurate determination of in situ temperatures, and established Site C0023 (32°22.0018′N, 134°57.9844′E, 4776-m water depth; fig. S1) in the vicinity of the previous drill site [(*13*); see supplementary materials]. Rigorous precautions during sampling and improvements in cell enumeration techniques increased the sensitivity in cell detection by five orders of magnitude compared with the previous study (*12*). For the quantification of cells that can be stained by a fluorescent dye (hereafter termed vegetative cells), the procedural blank was 4.2 ± 4.0 cells cm^{-3} of sediment (N = 20), thereby yielding a minimum quantification limit (MQL) of 16 cells cm^{-3}. Temperature measurements in the borehole constrained a steady-state temperature profile with a gradient of 110°C km^{-1} and a temperature of 120° ± 3°C in the deepest core retrieved from the basement at 1177 m below seafloor (mbsf) (figs. S3 and S4). The combination of authigenic minerals and thermally altered biomarkers reveals a history of episodic, short-term ingression of ~140° to 220°C hot hydrothermal fluids along permeable strata in the underthrust domain [(*14*); fig. S2].

At Site C0023, the depth profile of cell concentrations deviates notably from the global

[1]Center for Marine Environmental Sciences (MARUM), University of Bremen, Bremen, Germany. [2]Research and Development Center for Ocean Drilling Science, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Yokohama, Japan. [3]Kochi Institute for Core Sample Research, JAMSTEC, Kochi, Japan. [4]Center for Deep Earth Exploration (CDEX), JAMSTEC, Yokohama, Japan. [5]Graduate School of Oceanography, University of Rhode Island, Narragansett, RI, USA. [6]Department of Earth, Planetary, and Space Sciences, Department of Atmospheric and Oceanic Sciences, University of California, Los Angeles (UCLA), Los Angeles, CA, USA. [7]Center for Geomicrobiology, Department of Bioscience, Aarhus University, Aarhus, Denmark. [8]Faculty of Science and Technology, Kochi University, Kochi, Japan. [9]Department of Geology and Petroleum Geology, School of Geosciences, University of Aberdeen, Aberdeen, UK. [10]Department of Biological Sciences, University of Calgary, Calgary, AB, Canada. [11]Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany. [12]Institute for Extra-cutting-edge Science and Technology Avantgarde Research, JAMSTEC, Yokosuka, Japan. [13]Graduate School of Integrated Basic Sciences, Nihon University, Tokyo, Japan. [14]Geomicrobiology Research Group, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan. [15]Department of Environmental Systems Science, ETH Zürich, Zürich, Switzerland. [16]School of Geography, Earth and Environmental Sciences, Faculty of Science and Engineering, Plymouth University, Plymouth, UK. [17]Department of Earth and Planetary Sciences, Washington University in St. Louis, St. Louis, MO, USA. [18]Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA, USA. [19]Atmosphere and Ocean Research Institute, University of Tokyo, Tokyo, Japan. [20]Department of Subsurface Geobiological Analysis and Research, JAMSTEC, Yokosuka, Japan. [21]School of Earth Sciences, University of Queensland, St. Lucia, QLD, Australia. [22]Department of Earth Sciences, University of Toronto, Toronto, ON, Canada. [23]Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, MA, USA. [24]Department of Oceanography, Texas A&M University, College Station, TX, USA. [25]Department of Mathematical Science and Advanced Technology, JAMSTEC, Yokosuka, Japan. [26]Department of Earth System Sciences, Yonsei University, Seoul, Republic of Korea. [27]Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, Zürich, Switzerland. [28]GFZ German Research Centre for Geosciences, Potsdam, Germany. [29]Earthquake Research Institute, University of Tokyo, Tokyo, Japan.

*These authors contributed equally to this work. †Present address: Institute for Marine-Earth Exploration & Engineering (MarE3), JAMSTEC, Yokohama, Japan. ‡Present address: Kochi Institute for Core Sample Research, JAMSTEC, Kochi, Japan. §Present address: Department of Ecological Microbiology, University of Bayreuth, Bayreuth, Germany. ¶Present address: Queen Mary University of London, School of Geography, London, UK. #Present address: Graduate School of Engineering, Kyoto University, Kyoto, Japan. **Present address: Robert Koch-Institut, Berlin, Germany. ††Present address: School of Earth Sciences, University of Melbourne, Melbourne, VIC, Australia. ‡‡Present address: Department of Ecology & Environmental Studies, The Water School, Florida Gulf Coast University, Fort Myers, FL, USA. §§Present address: Department of Earth and Atmospheric Sciences, The University of Alberta, Edmonton, AB, Canada. ¶¶Present address: Department of Marine Sciences, University of Gothenburg, Gothenburg, Sweden. ##Present address: ExxonMobil, 22777 Springwoods Village Parkway, Spring, TX, USA. ***Present address: Graduate School of Geoscience, Kobe University, Kobe, Japan. †††Present address: Department of Oceanography, Pusan National University, Busan, Republic of Korea.

‡‡‡Corresponding author. Email: khinrichs@uni-bremen.de

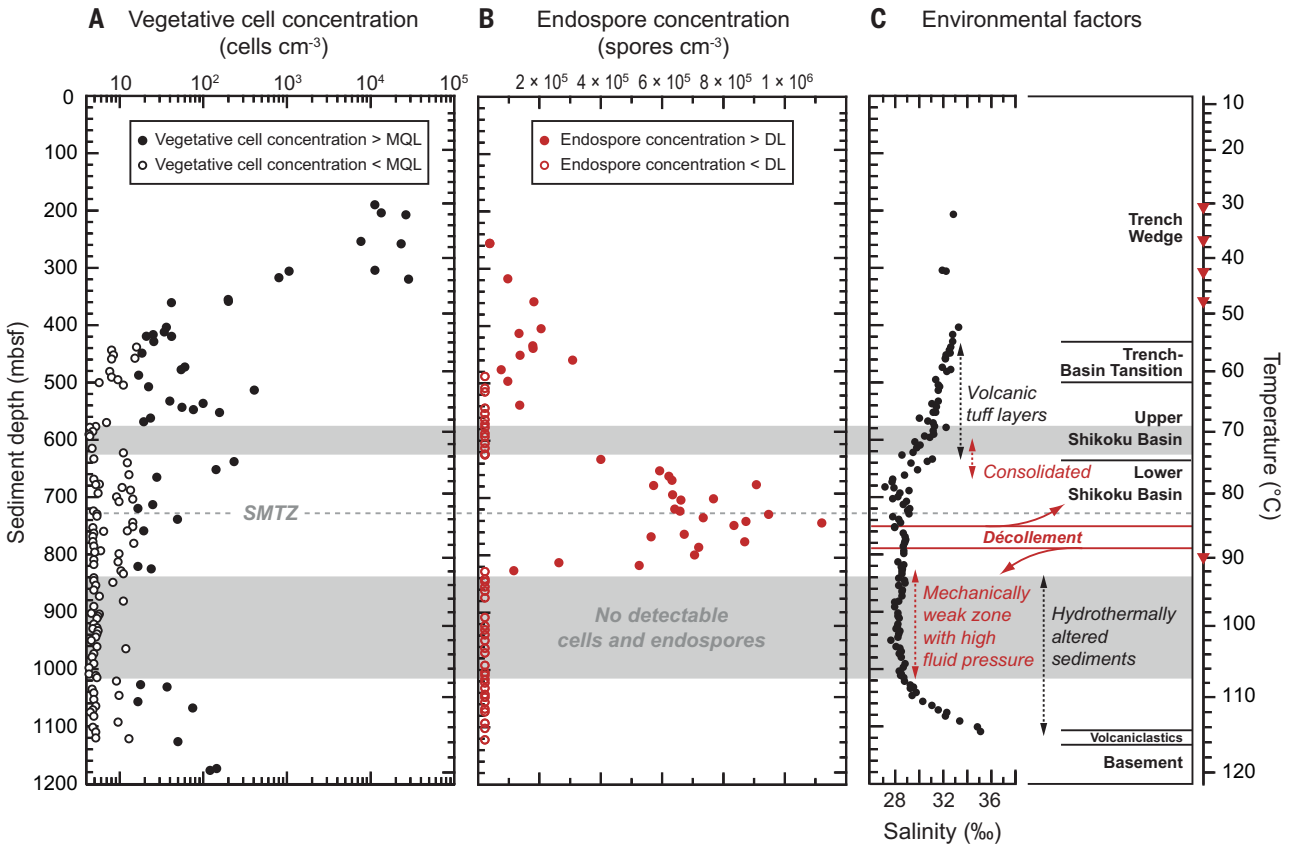

Fig. 1. Depth profiles of vegetative cells and endospores in relation to environmental factors at IODP Site C0023. (**A**) Concentrations of vegetative cells determined by counting of microbial cells fluorescently stained with SYBR Green I. (**B**) Concentrations of bacterial endospores derived from analysis of the diagnostic biomarker DPA; analytical sensitivity corresponds to a detection limit (DL) of 2.2×10^4 endospores cm^{-3}. (**C**) A schematic summary of temperature, tectonic units, and salinity showing the geochemical influence of basalt alteration in the basement; red symbols on the temperature axis designate the depth horizons where in situ temperature measurements were made. Gray shading indicates zones where concentrations of both vegetative cells and endospores were undetectable in all samples; the gray dashed line indicates the location of the SMTZ (compare Fig. 2).

trend of gradually decreasing cell concentrations observed in similarly deep but substantially colder (<30°C) sediments (*1*, *2*). At ~300 to 400 mbsf, concentrations of vegetative cells drop abruptly by two orders of magnitude and approach the MQL as temperature rises from 40° to 50°C (Fig. 1A). Concurrently, concentrations of endospores—that is, dormant, resistant structures affiliated with the bacterial phylum Firmicutes (fig. S5), which are widely found in marine sediments and soils (*15*, *16*)—increase to 2×10^5 cm^{-3} (Fig. 1B). Nevertheless, a small microbial population persists at >50°C in the form of both vegetative cells and endospores (Fig. 1). Down to the 120°C hot basement, sediments harboring microbial communities with up to 400 vegetative cells cm^{-3} are interspersed with intervals of up to 192-m thickness, in which no cells were detected (Fig. 1A and fig. S6). We rule out the possibility that the detection of cells resulted from contamination because cell concentration is neither related to the abundance of fractures in sediment cores nor related to the concentration of the perfluorocarbon-based contamination tracer supplied during the drilling operation (fig. S7); such relationships would be expected if contaminant cells were introduced by drilling fluids. Consistent with the extremely low concentrations of vegetative cells and the difficulty of extracting DNA from endospores (*17*), DNA yields were insufficient for producing reliable DNA-based community data for samples buried more deeply than 320 mbsf (*13*). In samples shallower than 320 mbsf, the community resembled those found in shallow subsurface sediments (*13*).

In contrast to the scattered distribution of vegetative cells in sediments >50°C, endospores show a clear zonation (Fig. 1B), as quantified by measurement of the diagnostic biomarker dipicolinic acid (DPA) (*18*). We rule out that substantial levels of DPA could have accumulated after the decay of endospores, given the propensity of 2-carboxylated pyridines to decarboxylate upon moderate short-term heating (*19*). Endospore concentrations rise prominently in a ~200-m interval of 75° to 90°C hot sediments, with a maximum of 1.2×10^6 endospores cm^{-3} at 85°C. The average endospore-to-vegetative cell ratio exceeds 6000 in sediments below 350 mbsf (table S1) and is thus two to three orders of magnitude higher than that in cold subseafloor sediments (*18*). Plausible scenarios for the accumulation of endospores in sediments that are nearly barren of vegetative cells relate to the thermal history of the site since the onset of trench conditions ~0.4 My ago (*12*, *14*) and involve the transitory growth of a thermophilic population of endospore formers [compare (*16*)] after temperature rose to ~50°C and its subsequent sporulation (fig. S8). Interestingly, in two expanded horizons, at 570 to 633 mbsf and 829 to 1021 mbsf, neither vegetative cells nor endospores were detected (Fig. 1 and fig. S6).

Pore-water profiles of microbial substrates and products provide evidence for microbial activity down to the ~16-My-old oceanic crust (Fig. 2). High concentrations of methane with a mean carbon isotopic composition ($\delta^{13}C$-CH_4)

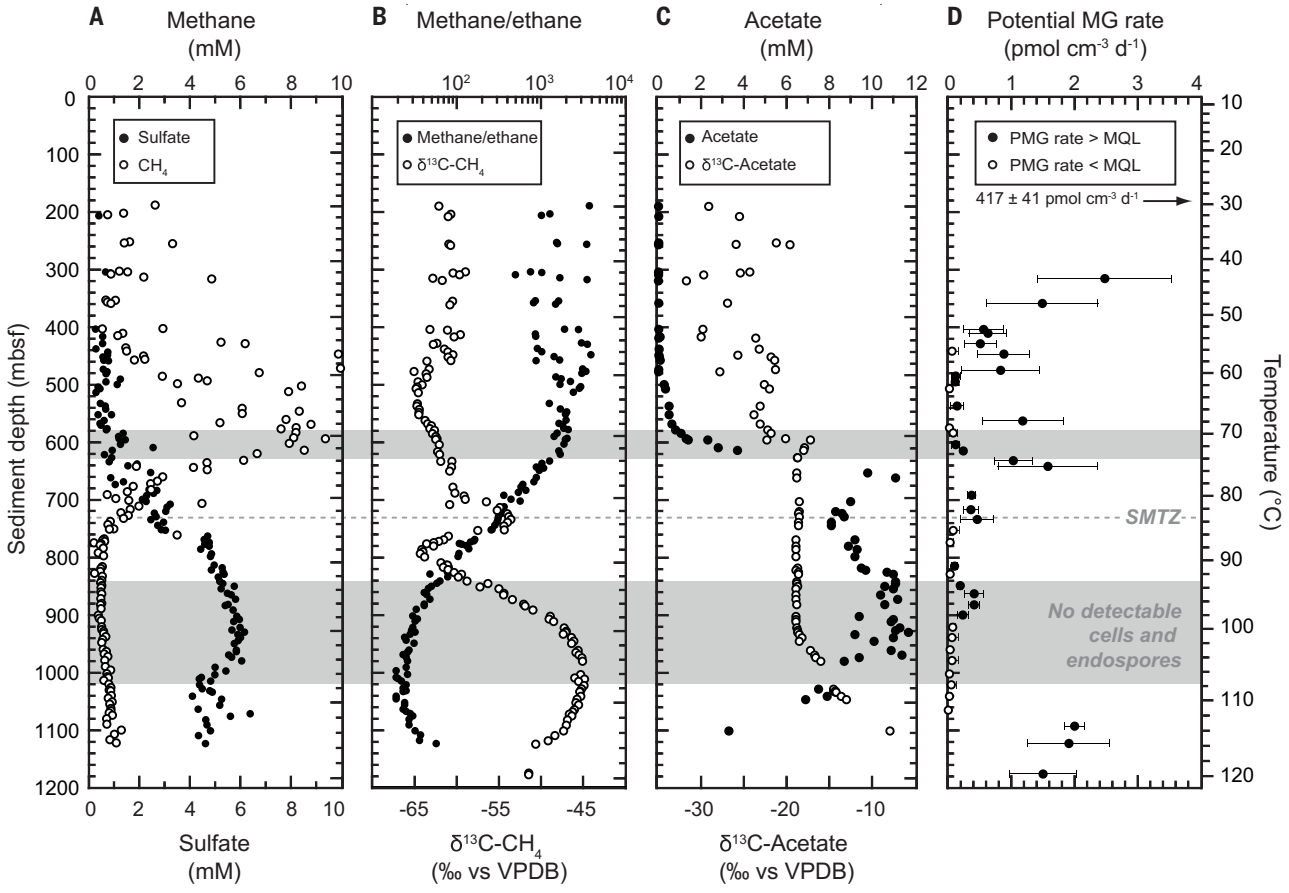

Fig. 2. Geochemical signals of microbial metabolism at Site C0023. (**A** to **D**) (A) Dissolved methane (*13*) and sulfate (*13*), (B) methane/ethane ratios (*13*) and δ^{13}C-CH_4, (C) dissolved acetate and δ^{13}C-acetate, and (D) potential rates of methanogenesis (MG) based on conversion of ^{14}C-CO_2 to ^{14}C-CH_4; note that the value at 180 mbsf is off-scale. Potential MG (PMG) rates were determined at 40°C for ≤360 mbsf, 60°C for 405 to 585 mbsf, 80°C for 604 to 775 mbsf, and 95°C for ≥816 mbsf. The MQL was 0.094 pmol CH_4 cm^{-3} day^{-1}. Gray shading, SMTZ, and the temperature axis are as in Fig. 1. VPDB in (B) and (D) is the Vienna Pee Dee Belemnite standard. In (D), error bars represent the standard deviation of three replicates.

of −61.3 ± 3.0 per mil (‰) (Fig. 2, A and B) indicate biogenic methanogenesis at least down to the 80° to 85°C hot sulfate methane transition zone (SMTZ) at ~730 mbsf. The positive excursion in δ^{13}C-CH_4 in the SMTZ (Fig. 2B) points to a biogenic methane sink and is consistent with previous observations from cultivation-based approaches that demonstrated the activity of thermophilic anaerobic methane-oxidizing communities at these temperatures (*20*, *21*). Below the SMTZ, methane is only present in micromolar concentrations, with rising δ^{13}C-CH_4 values and decreasing methane/ethane ratios indicating a relative increase of thermogenic hydrocarbons (Fig. 2B). Notably, a reversal of this trend at >1000 mbsf hints at a biogenic methane source above 100°C.

Diffusive profiles of pore-water constituents do not allow the distinction between current and recent in situ biogeochemical processes, whereas radiotracer experiments specifically target on-going microbial activity, albeit with some unavoidable deviation from in situ conditions. At Site C0023, radiotracer experiments reveal present-day methanogenic activity in 65% of the investigated samples (Fig. 2D). Potential rates of methanogenesis via CO_2 reduction in sediments below 300 mbsf are generally below 4 pmol cm^{-3} day^{-1} and thus within the range of previous observations made in the deep subseafloor (*22*). Their depth distribution is consistent with cellular concentrations (Fig. 1) and activities deduced from the pore-water profiles of methane (Fig. 2, A and B). Rates are highest in the methanic zone, decrease distinctly to <0.6 pmol cm^{-3} day^{-1} below the SMTZ, and drop to undetectable levels in 63% of the samples taken from the deep expanded horizon with no detectable cells and endospores (Fig. 2D). Notably, potential methanogenesis rates rise again to values observed in the methanic zone in the three deepest samples (Fig. 2D), thus confirming the existence of active methanogenic communities in 110° to 120°C hot sediments and pillow basalts above the basement.

Acetate is a key microbial substrate, and its generation from sedimentary organic matter upon heating has been suggested to fuel microbial life in deeply buried sediments (*23*). Throughout the sediment column of Site C0023, reactions degrading acetate via sulfate reduction and methanogenesis are exergonic, with Gibbs free energy yields becoming increasingly negative with depth (fig. S9). The concentrations of acetate and its carbon isotopic compositions (δ^{13}C-acetate) (Fig. 2C) indicate distinct changes in acetate utilization with temperature and depth. In the up-to-60°C hot upper 600 mbsf, low concentrations of acetate around 26 ± 22 μM (N = 19) are consistent with a steady state governed by tightly coupled microbial production and consumption, as observed in other sedimentary environments. The fluctuation of δ^{13}C-acetate around its average of −25.5 ± 3.4‰ implies ongoing metabolic activity (*24*). In sharp contrast, acetate utilization is minimal at 60° to 100°C. At 60° to 75°C, acetate concentrations rise steeply with the simultaneous decline of methane concentrations and accumulation of endospores, suggesting that microbial

consumption is no longer balancing the release of acetate from sedimentary organic matter. Nevertheless, a local minimum in acetate concentration at the SMTZ (Fig. 2C) is consistent with some microbial utilization at this geochemical interface. Below the SMTZ, acetate concentrations level at 9.2 ± 2.4 mM with an invariable δ^{13}C-acetate around −18.8 ± 0.5‰. The combination of high concentration and low isotopic variability implies an acetate pool without substantial turnover within the endospore-dominated zone as well as in the underlying 200-m-thick zone, where neither cells nor endospores were detected.

At >1030 mbsf, however, acetate concentrations decline and δ^{13}C-acetate monotonically increases with depth, reaching a maximum of −7.9‰ in the deepest pore-water sample recovered from 1101 mbsf. This trend is consistent with active hyperthermophiles degrading preferentially ^{13}C-depleted acetate, leaving the residual acetate isotopically enriched. Without continued consumption, diffusion would homogenize δ^{13}C-acetate variations, as observed in the overlying sediments. The drawdown of the acetate pool requires isotopic fractionation factors of −7.7 to −15.4‰ (fig. S10), which are consistent with those observed in lab cultures (*25*). The size of the sink would have to be on the order of 5×10^{-12} mol cm^{-3} year^{-1}. Given cellular concentrations of 10 to 100 cm^{-3} in sediments corresponding to this acetate sink, the required cellular metabolic rates are two to three orders of magnitude lower than those observed in lab cultures of the hyperthermophilic archaea *Pyrococcus furiosus* (*26*) and *Archaeoglobus fulgidus* (*27*) but two to three orders higher than in situ rates in deep sediments with temperatures <30°C (*28*). Thus, acetate profiles are consistent with the existence of a small acetate-utilizing microbial community at >100°C and suggest that the microbes at this high temperature require more energy and therefore turn over substrates faster than at lower temperature (*8*). Syntrophic acetate oxidation coupled to consumption of the resulting CO_2 and electrons by methanogens is a known acetate sink in deep sediments (*29*) and is considered to be particularly important at increased temperatures (*30*). This process is exergonic under in situ conditions (fig. S9) and could account for the increased methanogenesis rates (Fig. 2D) and the isotopic signature of methane (Fig. 2B) in the deepest portion of the borehole.

Our findings reveal the impact of increasing temperature with depth on microbial life. This is exemplified in the massive collapse of the population of vegetative cells in <0.4-My-old sediments at 300 to 400 mbsf. In this interval, temperatures of 40° to 50°C are within the upper growth range of mesophiles. The coincident accumulation of endospores as a result of a putative sporulation of mesophilic endospore-forming Firmicutes (Fig. 1) supports the conclusion that the abundance of microbial populations is primarily controlled by temperature-dependent physiological factors down to 600 mbsf. In the deeper portion of Site C0023, geological processes may exert additional control. A sharp decline in biogenic methanogenesis and acetate utilization at 70° to 75°C coincides with the upper growth range of thermophiles, but notably, this depth interval concurrently spans the lithological boundary between Upper and Lower Shikoku Basin (compare Fig. 1). At this boundary, tuffs (indurated volcanic ash) cease to be present. Tuff alteration forms smectite, and microbial reduction of Fe(III) in smectite serves as an energy-yielding process and has indeed been found to promote smectite-to-illite conversion at 500 to 600 mbsf at Site C0023 (*31*). Thus, a modulation of some types of microbial activity by microbe-mineral interactions is conceivable. Peak endospore concentrations at 85°C coincide with both the SMTZ and the plate boundary décollement. In this zone, brief periods of frictional heating during differential plate motion (*32*) likely cause additional challenges for microorganisms, but endospores and high acetate concentrations may provide a seed bank and energy, respectively, for an ecosystem recovery from episodic perturbations.

In the upper 200 m of the underthrust domain, at ~90° to 100°C, an expanded zone without detectable cells and with no geochemical signs of microbial activity traverses the sparsely populated sediments (Figs. 1 and 2). In this zone, undercompacted and mechanically weak sediments are overpressurized and affected by ~145° to 220°C hot fluids for short durations (*14*, *33*). The transient heating events may have locally sterilized sediment (*14*), but microbial cells, acetate consumption, and methanogenic activity prevail again in >100°C sediments, where mechanical strength and salinity increase toward the sediment-basement interface (Figs. 1 and 2 and fig. S2). Hydraulic communication between basalts and overlying sediment is evidenced by shared styles of epigenetic mineralization in the form of calcite veins and ferruginous metal oxides. Mass transfer between basal sediment and a basalt-hosted aquifer would increase the habitability of the basal sediment by reducing formation fluid pressure and by replenishing otherwise depleted substrates such as reduced iron and sulfate (*34*).

Our study reveals the dependence of microbial abundance and activity to critical temperatures around 40° to 50°C and 70°C; it moreover shows that life in the deep subseafloor is not constrained by an upper temperature limit below 120°C. Our findings highlight the interplay of geological processes, temperature, and microbial life in the deep, hot sediments of the Nankai Trough and suggest a critical influence of subduction-related geological processes on habitability.

REFERENCES AND NOTES

1. J. Kallmeyer, R. Pockalny, R. R. Adhikari, D. C. Smith, S. D'Hondt, *Proc. Natl. Acad. Sci. U.S.A.* **109**, 16213–16216 (2012).
2. R. J. Parkes *et al.*, *Mar. Geol.* **352**, 409–425 (2014).
3. F. Inagaki *et al.*, *Science* **349**, 420–424 (2015).
4. D. E. LaRowe, E. Burwicz, S. Arndt, A. W. Dale, J. P. Amend, *Geology* **45**, 275–278 (2017).
5. K. Takai *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 10949–10954 (2008).
6. J. P. Amend, E. L. Shock, *FEMS Microbiol. Rev.* **25**, 175–243 (2001).
7. M. A. Lever *et al.*, *FEMS Microbiol. Rev.* **39**, 688–728 (2015).
8. M. H. Møller *et al.*, *Front. Microbiol.* **9**, 967 (2018).
9. A. Wilhelms *et al.*, *Nature* **411**, 1034–1037 (2001).
10. I. M. Head, D. M. Jones, S. R. Larter, *Nature* **426**, 344–352 (2003).
11. G. F. Moore, A. Taira, A. Klaus, Expedition 190 Scientists, in *Proceedings of the Ocean Drilling Program, Initial Reports* (Ocean Drilling Program, College Station, TX, 2001), vol. 190.
12. B. Horsfield *et al.*, *Earth Planet. Sci. Lett.* **246**, 55–69 (2006).
13. V. B. Heuer, F. Inagaki, Y. Morono, Y. Kubo, L. Maeda, Expedition 370 Scientists, "Temperature limit of the deep biosphere off Muroto" in *Proceedings of the International Ocean Discovery Program* (International Ocean Discovery Program, College Station, TX, 2017), vol. 370.
14. M.-Y. Tsang *et al.*, *Mar. Pet. Geol.* **112**, 104080 (2020).
15. T. Aüllo, A. Ranchou-Peyruse, B. Ollivier, M. Magot, *Front. Microbiol.* **4**, 362 (2013).
16. C. Hubert *et al.*, *Science* **325**, 1541–1544 (2009).
17. S. Filippidou *et al.*, *Comput. Struct. Biotechnol. J.* **13**, 299–306 (2015).
18. L. Wörmer *et al.*, *Sci. Adv.* **5**, eaav1024 (2019).
19. A. R. Katritzky, A. R. Lapucha, M. Siskin, *Energy Fuels* **4**, 506–510 (1990).
20. J. Kallmeyer, A. Boetius, *Appl. Environ. Microbiol.* **70**, 1231–1233 (2004).
21. T. Holler *et al.*, *ISME J.* **5**, 1946–1956 (2011).
22. R. J. Parkes, B. A. Cragg, P. Wellsbury, *Hydrogeol. J.* **8**, 11–28 (2000).
23. P. Wellsbury *et al.*, *Nature* **388**, 573–576 (1997).
24. V. B. Heuer, J. W. Pohlman, M. E. Torres, M. Elvert, K.-U. Hinrichs, *Geochim. Cosmochim. Acta* **73**, 3323–3336 (2009).
25. D. Goevert, R. Conrad, *FEMS Microbiol. Ecol.* **71**, 218–225 (2010).
26. S. H. Brown, R. M. Kelly, *Appl. Environ. Microbiol.* **55**, 2086–2088 (1989).
27. D. E. Canfield, K. S. Habicht, B. Thamdrup, *Science* **288**, 658–661 (2000).
28. G. Z. Wang, A. J. Spivack, S. Rutherford, U. Manor, S. D'Hondt, *Geochim. Cosmochim. Acta* **72**, 3479–3488 (2008).
29. F. Beulig, H. Røy, C. Glombitza, B. B. Jørgensen, *Proc. Natl. Acad. Sci. U.S.A.* **115**, 367–372 (2018).
30. J. Dolfing, S. R. Larter, I. M. Head, *ISME J.* **2**, 442–452 (2008).
31. J. Kim *et al.*, *Geology* **47**, 535–539 (2019).
32. P. M. Fulton *et al.*, *Science* **342**, 1214–1217 (2013).
33. Y. Hamada *et al.*, *Prog. Earth Planet. Sci.* **5**, 70 (2018).
34. M. E. Torres *et al.*, *Geobiology* **13**, 562–580 (2015).
35. V. B. Heuer *et al.*, Dataset: Microbial abundance and activity at IODP Site C0023, Nankai Trough subduction zone. PANGAEA (2020); https://doi.org/10.1594/PANGAEA.923088.

ACKNOWLEDGMENTS

This research used samples and data provided by the International Ocean Discovery Program (IODP). The authors thank IODP and the Ministry of Education, Culture, Sports, Science, and Technology of Japan (MEXT) for providing an opportunity to explore the deep biosphere during Expedition 370. The expedition comprised simultaneous offshore and onshore work, conducted on the drilling vessel *DV Chikyu* and in the Kochi Core Center (KCC), respectively. We thank all operational and technical staff members on *Chikyu* and at KCC. In particular, we thank K. Akiyama, K. Aoike, N. Eguchi, M. Kyo, Y. Namba, and T. Sugihara for their knowledgeable support during project design; T. Saruhashi, I. Sawada, and T. Ikawa for successful drilling operations; and the Marine Works Japan, Co. for their technical support. We also thank K. Hagino and N. Tomioka at KCC for their contributions to sediment-age determination and electron microscopic

observations, respectively. This is a contribution to the Deep Carbon Observatory (DCO) and the Earth 4D: Subsurface Science and Exploration, CIFAR. **Funding:** This work was supported in part by the Japan Society for the Promotion of Science (JSPS) Strategic Fund for Strengthening Leading-Edge Research and Development (to JAMSTEC and F.I.); the JSPS Funding Program for Next Generation World-Leading Researchers (GR102 to F.I.); JSPS Grants-in-Aid for Scientific Research JP26251041, JP19H05503, and JP20K20429 (to F.I. and T.Ho.) and JP19H00730 (to Y.M., A.I., and T.Ho.); the Deutsche Forschungsgemeinschaft through projects 387745511 (to V.B.H.), 408178672 (to F.S.), 408249062 (to J.K.), 279667358, Hi 616-14-1 (to K.-U.H.), and through the Cluster of Excellence "The Ocean Floor – Earth's Uncharted Interface" (project 390741603), the IODP U.S. Science Support Program (National Science Foundation prime award OCE-1450528 to T.T.), and the Natural Environment Research Council awards NE/P015182/1 and NE/R003408/1 (to S.A.B. and H.M., respectively). Additional support enabling this project was provided by the DCO. **Author contributions:** K.-U.H., F.I., V.B.H., and Y.M. designed the research; V.B.H., F.I., and Y.M. lead IODP Expedition 370 as co-chief scientists; Y.K. and L.M. coordinated research operations during IODP Expedition 370; V.B.H., F.I., Y.M., A.J.S., B.V., T.T., F.B., F.S., S.T., S.A.B., M.C., S.H., T.Hi., K.H., T.Ho., A.I., H.I., N.K., M.Ka., L.L., H.M., H.-L.M., K.M., N.O., D.P., M.J.R., J.S., M.-Y.T., D.T.W., E.W., Y.Yamam., K.Y., R.R.A., C.G., Y.H., J.K., J.W., and L.W. performed research; Y.Yamad. and M.Ki. provided crucial methods; V.B.H., F.I., Y.M., A.J.S., B.V., T.T., F.B., F.S., S.T., L.W., and K.-U.H. analyzed data; and V.B.H. and K.-U.H. wrote the paper with input from all authors. **Competing interests:** None. **Data availability:** All shipboard data are documented and are openly accessible in the IODP Expedition 370 Proceedings (*13*), with references to the respective tables and figures given in the supplementary materials. All shore-based data are accessible in the PANGAEA database (*35*).

SUPPLEMENTARY MATERIALS

science.sciencemag.org/content/370/6521/1230/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S10
Tables S1 and S2
References (*36*–*83*)
MDAR Reproducibility Checklist

View/request a protocol for this paper from *Bio-protocol*.

15 July 2020; accepted 13 October 2020
10.1126/science.abd7934

Science

Temperature limits to deep subseafloor life in the Nankai Trough subduction zone

Verena B. Heuer, Fumio Inagaki, Yuki Morono, Yusuke Kubo, Arthur J. Spivack, Bernhard Viehweger, Tina Treude, Felix Beulig, Florence Schubotz, Satoshi Tonai, Stephen A. Bowden, Margaret Cramm, Susann Henkel, Takehiro Hirose, Kira Homola, Tatsuhiko Hoshino, Akira Ijiri, Hiroyuki Imachi, Nana Kamiya, Masanori Kaneko, Lorenzo Lagostina, Hayley Manners, Harry-Luke McClelland, Kyle Metcalfe, Natsumi Okutsu, Donald Pan, Maija J. Raudsepp, Justine Sauvage, Man-Yin Tsang, David T. Wang, Emily Whitaker, Yuzuru Yamamoto, Kiho Yang, Lena Maeda, Rishi R. Adhikari, Clemens Glombitza, Yohei Hamada, Jens Kallmeyer, Jenny Wendt, Lars Wörmer, Yasuhiro Yamada, Masataka Kinoshita and Kai-Uwe Hinrichs

Science **370** (6521), 1230-1234.
DOI: 10.1126/science.abd7934

Deep, hot, and more alive than we thought

Marine sediments represent a massive microbial ecosystem, but we still do not fully understand what factors shape and limit life underneath the seafloor. Analyzing samples from a subduction zone off the coast of Japan, Heuer *et al.* found that microbial life, in particular bacterial vegetative cells, decreases as depth and temperature increases down to ~600 meters below the seafloor, corresponding to temperatures of ~70°C. Below this limit, endospores are common—a remnant, and a potential reservoir, of bacterial life. Deeper still is a sterile zone, and below 1000 meters is a scalding realm populated by vegetative cells. At such great depths, high concentrations of acetate and sulfate coexist, and there are also signs of hyperthermophilic methanogenesis. These data provide a fascinating window into an extreme and inhospitable environment that nonetheless supports microbial life.

Science, this issue p. 1230

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. The title *Science* is a registered trademark of AAAS.