ECOLOGY

Microbial dormancy in the marine subsurface: Global endospore abundance and response to burial

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Marine sediments host an unexpectedly large microbial biosphere, suggesting unique microbial mechanisms for surviving burial and slow metabolic turnover. Although dormancy is generally considered an important survival strategy, its specific role in subsurface sediments remains unclear. We quantified dormant bacterial endospores in 331 marine sediment samples from diverse depositional types and geographical origins. The abundance of endospores relative to vegetative cells increased with burial depth and endospores became dominant below 25 m, with an estimated population of 2.5×10^{28} to 1.9×10^{29} endospores in the uppermost kilometer of sediment and a corresponding biomass carbon of 4.6 to 35 Pg surpassing that of vegetative cells. Our data further identify distinct endospore subgroups with divergent resistance to burial and aging. Endospores may shape the deep biosphere by providing a core population for colonization of new habitats and/or through low-frequency germination to sustain slow growth in this environment.

INTRODUCTION

Since the 1990s, scientific drilling of the seafloor has revealed the abundance and relevance of microbial cells in subsurface sediments. Size estimates for the size of the sedimentary subseafloor biosphere have been based on direct counts of microbial cells (*1*, *2*) or on chemical approaches that target specific microbial biomarkers (*3*). Recent publications investigating a wide range of marine sediments and using refined analytical and computational methods have arrived at a number between 2.9×10^{29} (*2*) and 5.4×10^{29} (*4*) cells, corresponding to ~1% of Earth's total living biomass. These and previous figures are all based on the observation that cell and biomarker abundances decrease with sediment depth, as microbes face extreme environmental conditions, e.g., increased temperature, and ongoing starvation in an ecosystem gradually being depleted of available energy sources. However, microbial life has proven to be capable of withstanding such challenging conditions; recently, an active community in ~20–million year (Ma)–old coal beds located ~2.5 km below the seafloor has been described (*5*, *6*). This study (*5*) also observed a sharp drop in cell concentration at ~1.5 km below the sediment surface, possibly reflecting the increased cost of sustaining life at increasing temperatures.

While the limit of sediment habitability, and the question of whether temperature is the factor determining that limit, have yet to be resolved, more and more evidence suggests that survival strategies are implemented by cells during burial. Through burial, cells remain viable (*7*) but need to undergo extreme adaptations. Lever *et al.* (*8*) reviewed the wide range of specific traits observed in starved cultures and in severely energy-limited environments, such as reduction of both size and carbon content, changes in cell morphology and motility, modification of cell composition, and a decrease in minimum energy requirements. At the community level, survival in the deep biosphere also benefits from a shared effort to secrete enzymes that enable the turnover of more recalcitrant organic matter (*9*).

In addition to adaptations in their vegetative lifestyle, several bacterial strains have developed a unique strategy to overcome challenging environmental conditions, namely, conversion into specialized, metabolically quiescent, persistent, and resistant forms (*10*). This strategy is expressed in the formation of aerial spores, cysts, cyanobacterial akinetes, exospores, and endospores. Even Archaea are thought to produce dormant, spore-like cells (*11*). Among these resistance forms, bacterial endospores are the ones capable of facing the most challenging conditions such as intense heat and desiccation (*12*), and show extreme durability, having been described to remain viable over millions of years under certain conditions (*13*, *14*). The identification of an endospore-former, *Desulforudis audaxviator*, as the sole inhabitant of an isolated ecosystem situated several kilometers deep, below a South African gold mine (*15*), and the isolation of viable endospore-forming Firmicutes from deep sediment samples (*16*) or basaltic fluids (*17*) suggest the possibility of endospore survival over geological time scales in the deep biosphere.

The ability to form endospores is characteristic for many members of the large and highly diverse phylum Firmicutes and is broadly distributed among the various genera of this phylum. Numerous molecular studies of microbial diversity indicate that Firmicutes are not typically a dominant bacterial clade (e.g., *18*, *19*), findings perhaps explained by the facts that a considerable proportion of Firmicutes is likely present as endospores, which are resistant to cell lysis and DNA extraction (*20*, *21*). For Kawai *et al.* (*22*), this perspective explained why their studies of endospore-specific genes suggested that endospore-formers accounted for <10% of the microbial population at sites off Peru, even though previous studies had described endospores to be as abundant as vegetative cells in this system (*23*).

In contrast, Firmicutes consistently dominate cultures of microorganisms isolated from the marine subsurface (*16*, *24*–*26*). Considering this fact and the extraordinary robustness of endospores, we expect that endospore formation is an important survival strategy

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in the deep biosphere and that endospores could be broadly distributed in this habitat. However, the abundance and ecological role of endospores in the marine and terrestrial subsurface remain largely unknown. Owing to the establishment of dedicated, culture-independent analytical protocols (*27*) based on detection of the diagnostic endospore biomarker dipicolinic acid (DPA), a first glimpse into the potential importance of endospores was obtained: With increasing depth, endospores actually outnumbered vegetative cells in three sediment cores from the Peru Margin (*23*). Data from Aarhus Bay (*28*) and the North Atlantic (*29*) point to the conclusion—i.e., the decrease in abundance of these specialized, metabolically quiescent cells with depth is less pronounced than that of their vegetative counterparts.

Motivated by these observations, we aimed to assess the biomass of bacterial endospores in the marine subsurface on a global scale. We analyzed the concentration of endospores, based on DPA quantification, and of vegetative cells in more than 300 sediment samples from highly diverse and representative marine settings, including coastal and open-ocean sites, and samples from different provinces, depths, and ages. A set of independent modeling approaches was used to generate estimates of global endospore abundance, while availability of complementary data allowed us to evaluate the factors driving sedimentary endospore concentration and the response of this population to burial and aging.

RESULTS AND DISCUSSION

Downcore distribution of endospores and vegetative cells in the marine subsurface

The concentration of vegetative cells, for which genomic DNA is accessible and hence quantifiable with either microfluidic digital polymerase chain reaction (dPCR) or flow cytometry techniques, decreases with increasing depth (Fig. 1A) and thereby follows the typical trend of microbial cell concentrations observed in the subseafloor (*1*, *2*). The high sensitivity of the dPCR method allows quantification of low concentrations in the range of hundreds of cells per gram. When plotting vegetative cells versus depth on a log-log scale, a strong linear correlation can be observed (R^2 = 0.519, P < 0.001). The slope of this decrease is notably larger than the one described in the most recent global dataset (*4*), which may result not only from sample selection but also from offsets between cell counts and dPCR-based data (*30*). In marked contrast, a response to burial is less evident for DPA concentration as a proxy for the concentration of bacterial endospores: The linear correlation with depth is weak (Fig. 1B; R^2 = 0.136, P < 0.001), and endospore concentration decreases only slightly and remains relatively constant in a range between roughly 10^5 and 10^6 endospores/ml. Data from the North Atlantic, Peru Margin, and Aarhus Bay (*29*) showed similar patterns, with only a slight decrease of endospore concentration with depth. With the current extraction and analysis methods, one-third of our analyzed samples did not yield useable data, as DPA concentration was below our current detection limit (cf. Fig. 1B) or because of poor DPA recovery from the spiked sample.

To better understand the two contrasting dynamics of endospores and vegetative cells, we examined the ratio of these two cell types (Fig. 1C). Endospores contribute massively to the deep biosphere, and their relative concentrations exceed even previous estimates (*23*), which suggested that endospores could be as abundant as vegetative cells in deep sediments. While accounting for only about 0.1% of the vegetative cells in surface sediments, regression of the endospore/vegetative cell ratio versus depth indicates that already at 25 m below seafloor (mbsf) endospores become dominant. In deeply buried sediments, metabolically quiescent cells thus outnumber vegetative cells, and the potential presence of resistant forms different from endospores could tip the balance even further.

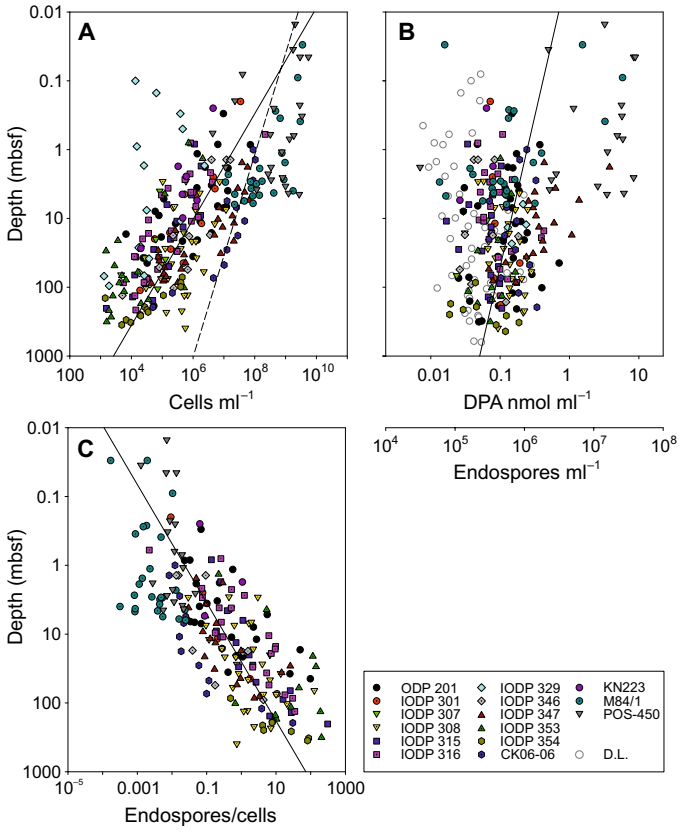

Fig. 1. Distribution of vegetative cells and endospores versus depth. Concentrations of (**A**) vegetative cells and (**B**) endospores and (**C**) ratio of endospores to vegetative cells versus sediment depth in samples from various Ocean Drilling Program (ODP) and Integrated Ocean Drilling Program (IODP) expeditions and national research cruises (cf. table S1). Cell concentrations are based on 16*S* rRNA gene targets quantified using dPCR data or on cell enumeration via flow cytometry (M84/1 and POS-450) and endospore concentrations on the diagnostic biomarker DPA. The solid regression line in (A) is log cells = 7.319 – 1.301 log depth (R^2 = 0.519, P < 0.001, n = 267) from this study, and the dashed line is the regression line from the global estimate of the marine subseafloor biosphere (*4*): log cells = 8.05 – 0.68 log depth (R^2 = 0.70, n = 2037). The regression line in (B) is log endospores = 6.039 – 0.228 log depth (R^2 = 0.136, P < 0.001, n = 220). Samples with DPA concentration below our current detection limit (D.L.) are shown as empty circles. These data are not included in the regression. The regression line in (C) is log (endospores/cells) = −1.636 + 1.164 log depth {R^2 = 0.577, P < 0.001, n = 199; n < 220 [number plotted in (B)] because cell concentration data are not available for all samples in which endospores were detected}.

The high abundance of endospores in the deep biosphere is consistent with the fact that (i) Firmicutes often dominate microbial cultures of samples isolated from the subseafloor (*16*, *24*–*26*), although sporulation has not been shown for all Firmicutes genera isolated from these systems, and (ii) endospore-formers have been identified as one of the populations whose relative abundance increased with depth in a collection of 16*S* ribosomal RNA (rRNA) amplicon sequence libraries from marine sediments (*31*). A major contribution of fossil DPA to the biomarker pool is not expected, as this compound is very quickly

released from the spore core during the initial stage of germination (*32*) or after cell inactivation (*33*), and it is rapidly degraded afterward (*34*); the same dynamics are also seen in soil slurries (*35*) or under anoxic conditions (*36*). If preservation of fossil DPA played an important role in our dataset, then we would expect to observe the highest concentrations under conditions conducive to organic matter preservation, i.e., in sediments with the highest concentrations of total organic carbon (TOC). However, the fact that no relationship between DPA concentration and TOC is observed argues against preservation contributing to the increase of DPA relative to vegetative cells (section S1 and fig. S1).

Factors controlling endospore abundance in the marine subsurface

A number of sites, mainly from the brackish Baltic (IODP 347) and Black Seas (M84/1) and from the Rhone Delta (POS-450), show higher concentrations of DPA in the upper sediment and a stronger decrease with depth. Most of these samples share one other characteristic: They were obtained from relatively recent sediments. When plotting DPA-derived endospore concentration against estimated sediment age (Fig. 2A), two subgroups can be identified: a number of younger samples, in which endospore concentration decreases quickly with depth, and a group of older samples, in which concentration remains rather constant. Segmented linear regression ($R^2 = 0.656$, $P < 0.0001$) defines a breakpoint at an age of ~14.2 thousand years. On the basis of this regression, during the time span of 1.4×10^4 years in segment 1, endospore concentration decreases by more than two orders of magnitude, while in the 2×10^7 years represented by segment 2, endospore concentration is only halved. This rapid decrease of endospore concentration in younger near-surface sediments and the relatively stable concentration across depth in older sediments suggest a bimodal distribution of potential longevity and thus the existence of at least two different endospore subpopulations.

Culture data investigating the effects of exposure of endospores to increasing temperatures are useful in assessing their potential for long-term survival, as higher temperature increases the costs of sustaining life and consistently accelerates inactivation. Consequently, survival at increasing temperature is a proxy for endospore stability. Plotting the time required to reduce an endospore population by one order of magnitude (decimal reduction value, *D*) on a log scale against temperature typically reveals a negative linear correlation (*37*). Nicholson (*38*) compiled such thermal inactivation studies, extrapolated these kinetics to ambient temperatures, and observed clearly separated groups of endospores (Fig. 2B). The most persistent group was formed by the two strains of thermophilic endospores investigated (group 3), while *D* values were substantially lower for the two groups of mesophilic cultures (groups 1 and 2).

In the present study, burial and the associated aging and geothermal heating (blue symbols in Fig. 2B) place the investigated sediments in a regime in which the endospores are aged enough to have suffered notable losses from the most labile subpopulation (analogous to group 1, $D = 150$ years at 5°C). Meanwhile, the more resistant subpopulations would remain mostly unaffected, and only the

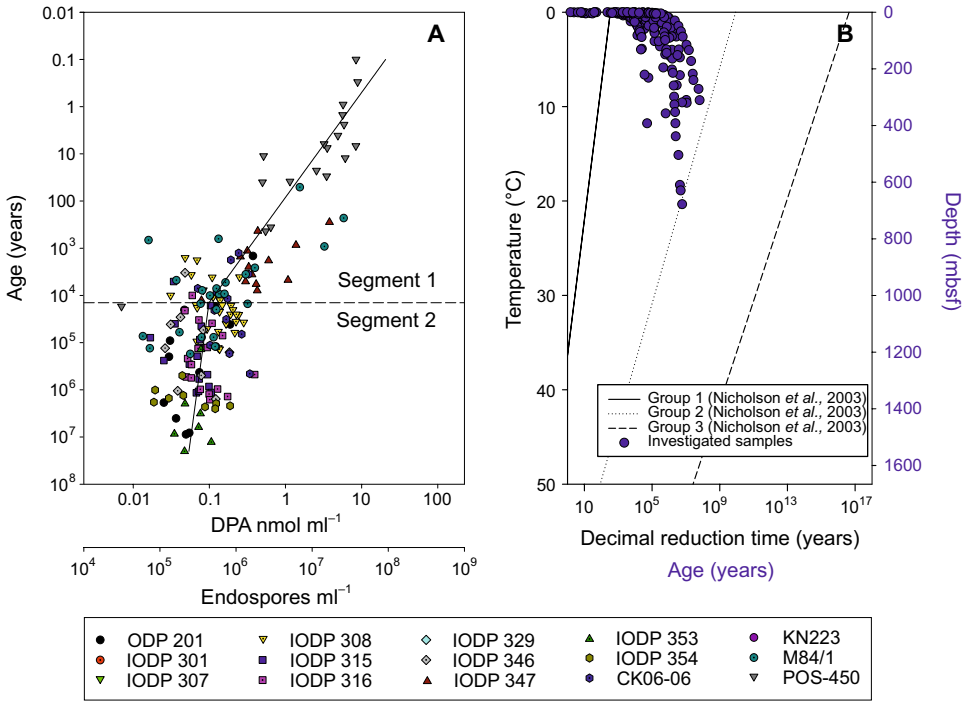

Fig. 2. Endospore response to burial and aging. (**A**) Endospore concentration versus sediment age in samples from various ODP and IODP expeditions and national research cruises (cf. table S1). The segmented regression line ($R^2 = 0.656$, $P < 0.0001$, $n = 166$, solid line) in (A) is composed of a first section (age < 14,266 years) with log endospores = 7.515 − 0.450 log age and a second section (age > 14,226 years) with log endospores = 5.993 − 0.084 log age. The lower number of samples in comparison to Fig. 1B is because sediment age data were not available for all sites. (**B**) Lines represent temperature-dependent decimal reduction values for three groups of increasingly resistant endospores investigated in thermal inactivation experiments (*38*). They denote the time needed to reduce the endospore population by one order of magnitude at a given temperature. Blue dots indicate depth and age of samples analyzed in this study. Equivalence of depth and temperature axis is obtained by applying a geothermal gradient of 30°C/km.

deepest and oldest samples would be close to reaching the decimal reduction time for group 2 endospores.

Our data thus seem to indicate that a more ephemeral subpopulation of endospores can be deposited at the sediment surface. This subpopulation could be especially important in systems where a large amount of fresh terrestrial material can reach the seafloor, as is the case of the Rhone Delta or the freshwater-influenced Baltic and Black Sea. Germination under these relatively favorable conditions could be an additional factor in decimating the initial endospore population. The recorded time scales are also roughly the same order of magnitude as the ~350-year half-life for cultivable thermophilic endospores in recent sediments (<4500 years) from Aarhus Bay (*39*, *40*). Meanwhile, the pool of more stable endospores, possibly including many thermophiles, would resist and dominate in deeper sediments and in surface sediments with already aged organic material. A decimation of this population would require even deeper burial and the associated thermal stress. At the same time, these losses could be partially compensated by the heat- or starvation-driven sporulation of vegetative cells. Impressive evidence that strongly aged endospore populations remain viable was recently provided by incubation of autoclaved, 20-Ma-old coal beds from ~2 km depth, which revealed active microbial communities consistently dominated by Firmicutes (*6*). The described scenario fits the hypothesis recently formulated by Petro *et al.* (*31*): Microbial community assembly in marine sediments is driven by selection mechanisms, which filter populations from the surface and leave only a subset of microorganisms harboring favorable traits, as could be more stable endospores.

Estimates of global endospore abundance in the marine subsurface

To quantitatively assess the global endospore abundance in marine sediments, we chose a three-pronged approach based on methods previously used for cell count–based estimates of the marine deep biosphere (*1*, *2*, *4*). The first approach (average sediment depth calculation) previously used by Parkes *et al.* (*1*, *4*) applies a linear regression of log cells (in this case, log DPA or log endospores) versus log depth (cf. Fig. 1, A and B) and integrates calculated cell or endospore abundances along the average sediment thickness. The current dataset yields an endospore population of 8.2×10^{28} (1.8×10^{13} mol DPA) or 63% of the corresponding calculated vegetative cell population (1.3×10^{29}). The estimate for vegetative cells obtained from our dataset is lower than the earlier estimate of 5.4×10^{29} cells (*4*). The difference possibly arises from sample selection, with the relatively high proportion of open-ocean samples (24% of data; cf. fig. S2 and table S1), and from offsets between cell counts and dPCR-based data (*30*). Unfortunately, the log-log correlation of endospore concentration with depth is weak ($R^2 = 0.136$), limiting the robustness of this approach.

The second and third methods are based on the work of Kallmeyer *et al.* (*2*) who parameterized vegetative cell distribution at each studied site by adjusting downcore trends to a power curve. The parameters that described these curves were then correlated to mean sedimentation rate and distance from land. On the basis of those correlations, the geographical distribution and global abundance of subseafloor microbes could be modeled. Analogously, in our second approach (single-site parameterization), DPA-derived endospore concentration over depth at individual sites was adjusted to a power curve described by the variables endospore concentration at 1 m depth (b) and rate of decrease (m). From the 26 sites tested, data could be adjusted to a power curve in only 8, which reduces confidence in this model (fig. S3). Fortunately, these eight sites covered a large range of b (2.2×10^5 to 2.9×10^7 endospores ml^{-1}) and m (−0.17 to −0.94) values, and we were able to test how they correlated to several environmental parameters (table S2). Best fits for $\log(b)$ ($R^2 = 0.883$, $P < 0.001$; fig. S4A) and m ($R^2 = 0.832$ and $P = 0.002$; fig. S4A) were obtained with suspended particulate organic carbon (POC) concentrations obtained by remote sensing (*41*). As POC represents a mixed signal between in situ primary productivity and external input of organic detritus, these parameters may be positively influenced by both high aquatic productivity and riverine input.

Making use of the available map of POC concentration, endospore concentration at each node was modeled and integrated over the depth of habitable sediment. Because the marine deep biosphere seems more sensitive to heating than previously expected, with a major drop in cell concentration having been described already at temperatures of ~40°C (*5*), and because endospore concentration has been determined only to a depth of ~700 mbsf, we limited calculations to a maximum depth of 1000 m. We believe this to be more realistic than previously used (*2*, *3*), when temperature limits of 90° and 120°C and corresponding depths of 3000 and 4000 m were assumed. Summing up global abundance in the top kilometer yields a total DPA pool of 5.6×10^{12} mol. This pool size corresponds to 2.5×10^{28} endospores, which broadly agrees with the value obtained through summation of endospores in the average sediment thickness approach (8.1×10^{28}). Endospores accumulate in coastal regions and marginal seas, possibly fueled by a large input of endospores from terrestrial sources or from bacterial growth in high primary productivity areas. This observation is consistent with high concentrations observed in shallow sediments from the Aarhus Bay (*28*).

In the third approach (vegetative cell to endospore conversion), the robust correlation of the ratio between endospores and vegetative cells versus depth (Fig. 1C) was used to convert available cell concentrations obtained by the single-site parameterization (*2*) to downcore endospore concentration at each node. For the reasons explained above, also in this case we limited the depth of the habitable sediment to 1000 m; numerical integration of the calculated downcore curves of endospore concentration leads to the aerial abundance of endospores. Summing up global abundance yields a total DPA pool of 4.2×10^{13} mol, equivalent to 1.9×10^{29} endospores, which is almost one order of magnitude larger than the second approach and also larger than the corresponding vegetative cell population (8.5×10^{28}; Table 1).

The offset between the different modeling approaches can be explained by some intrinsic biases. The calculation based on average sediment thickness is limited by the poor regression and possibly underestimates endospore concentration, as the decreasing linear trend is mainly driven by the few samples with high values from the brackish Baltic and Black Sea and from the Rhone Delta. The approach based on single-site parameterization of endospores is based only on those sites in which a decrease of endospore concentration over depth, however small, could be observed. Thereby, the fact that for most sites no consistent decline could be observed is neglected, which potentially results in an underestimation of the total endospore population. The approach based on the vegetative cell to endospore conversion is hampered by the translation of the dPCR-based endospore/vegetative cell ratio to a cell count–based model (*2*), given potential analytical offsets (*30*). In addition, in coastal areas in which vegetative cell concentration decreases only slightly, this

Table 1. Estimates of endospore abundance and biomass. Summary of vegetative cell and endospore abundance for the top kilometer of subseafloor sediment based on the three modeling approaches performed. Biomass data are calculated from DPA abundance in the case of endospores and from cell abundance in the case of vegetative cells.

Modeling approach	Data used	Endospore population	Endospore biomass (Pg C)	Vegetative cell population	Vegetative cell biomass (Pg C)
Average sediment depth calculation	Global correlation of endospore concentration vs. depth (this study)	8.2×10^{28}	15	1.3×10^{29}	1.8
Single-site parameterization of endospore concentration	Endospore concentration vs. depth at single sites (this study)	2.5×10^{28}	4.6	–	–
Vegetative cell to endospore conversion	Global correlation of endospore/cell ratio vs. depth (this study). Modeled global abundance of vegetative cells vs. depth (*2*)	1.9×10^{29}	35	8.5×10^{28}	1.2

approach may model an unrealistically large increase of endospores over depth. Despite these caveats, the combination of the three approaches effectively narrows down the expected size of the subseafloor endospore population (Table 1).

Assuming a DPA content of ~10% of endospore dry weight (*32*), the calculated global DPA pool can be converted into endospore biomass. This calculation is independent from the size of individual endospores or the calculated size of the endospore population. The resulting range of 4.6 (single-site parameterization) to 35 Pg (vegetative cell to endospore conversion) is larger than the corresponding estimate for vegetative cell biomass of 1.2. to 1.8 Pg and is also larger than the estimate for 4000-m sediment thickness of 4.1 Pg (*2*). The difference may be explained by the small size of vegetative cells in the deep biosphere (*2*). If endospores are produced in the deep biosphere rather than inherited from shallower sediments, then their size and thereby DPA content could, in analogy to vegetative cells, decrease with burial depth; consequently, the estimate for absolute endospore numbers in the subseafloor would need to be corrected toward higher values. The endospore biomass estimate, however, would not be affected, as long as the relative endospore DPA content stays constant. Our data identify bacterial endospores as an enormous biomass pool that has been overlooked so far. Adding our endospore estimates (4.6 to 35 Pg C) to the recent census of biomass on Earth (~550 Pg C) (*42*), the total biomass adds up to be between 555 and 585 Pg C, with endospores in the marine subsurface contributing between 0.8 and 6%, depending on the modeling approach.

CONCLUSIONS

With this study, we have estimated the size of the endospore-forming, dormant deep-sea marine biosphere, and demonstrated that its biomass may exceed that of vegetative cells. Existence of additional pools of other specialized, dormant cells cannot be ruled out and remains to be investigated. The marine subsurface thus harbors an extremely large population of endospores that may serve as a seed bank, with their genomic and functional diversity remaining potentially available for long-time and far-distance transportation, germination, and colonization of new habitats (*43*). Burial may additionally serve as a selection process (*31*), as suggested by the existence of endospore populations with different inactivation dynamics or the ubiquity of the presumably more resistant thermophilic endospores (*39*, *44*, *45*). Laboratory experiments have substantiated the potential for temperature-triggered germination of a variety of sedimentary endospores (*44*, *46*, *47*). Gradual heating during burial might therefore lead to the outgrowth of parts of this population, as might the release of temperature-dependent substrates (*48*). Such sporadic awakening of endospores, either through detection of improved environmental conditions or stochastically [microbial scout hypothesis (*49*)], would translate into peaks of metabolic activity. Hence, germination events are expected to occur at a slow pace, and the associated energy consumption over time would be low. In this way, low-frequency (random) awakening would mimic extremely slow growth, as the one observed in the deep biosphere (*50*). The endospore pool may thus play an active role in the deep biosphere through ecological selection of more resistant endospores and selective or stochastic germination during burial, and also by shaping their chemical microenvironment (*51*). Similar to vegetative cells, the ultimate factor limiting survival of these specialized cells in the deep biosphere still needs to be resolved.

MATERIALS AND METHODS

A total of 331 sediment samples from 47 sites and with a maximal depth of 629 mbsf were analyzed for endospore and vegetative cell concentrations (table S1 and fig. S2). Endospores were quantified using the specific biomarker DPA. DPA was extracted from sediments by autoclaving and quantified by recording the fluorescence of the DPA-Tb complex following a protocol based on (*27*). Endospore numbers were calculated from DPA concentration by applying a DPA cell quota of 2.24×10^{-16} mol per endospore (*52*). This value is based on DPA cell quota from only six isolates (SD = 0.63×10^{-16} mol per endospore), and its robustness will benefit from future studies with additional isolates or investigating the effect of changing

environmental conditions on DPA content. Cell numbers were obtained by flow cytometry [M84/1 and POS-450 (*53*)] or by dPCR using microfluidics (*54*). dPCR-based gene abundance has been previously published (*30*). Complementing data (e.g., age and temperature) were obtained from cruise reports and available databases.

For the estimation of global endospore abundance, three methods were used. In the first case (average sediment depth calculation), the regression log endospores $ml^{-1} = 6.039 - 0.228$ log depth was used to calculate endospore concentration in discrete layers for the upper 721 m, corresponding to the averaged sediment thickness (*55*). Considering this layer thickness and global ocean area, total endospore numbers were calculated and summed up. For vegetative cells, the same approach was followed using log cells $ml^{-1} = 7.319 - 1.301$ log depth.

In the second case (single-site parameterization), individual downcore plots of endospore concentration were generated for each site; however, only those sites for which samples deeper than 10 m were available were considered. In selected sites, a decrease was observed and could be adjusted to a power curve (e.g., fig. S3). When such a downcore profile could be reconstructed ($R^2 > 0.3$), b was defined as the endospore concentration calculated for 1 m depth, and m was defined as the power-law rate of decrease. The variables b and m were then correlated to several environmental parameters: chlorophyll *a* concentration, distance from land, particulate inorganic carbon, POC, and sedimentation rate; the strongest correlations were obtained for POC concentration (table S2 and fig. S4). The GMT software (*56*) was used to construct a grid of aerial endospore abundance based on these correlations and the available maps for POC (*41*) and sediment thickness (*57*, *58*), and to calculate global abundance of endospores.

The third approach (vegetative cell to endospore conversion) was based on the estimate of the marine subseafloor microbial population by Kallmeyer *et al.* (*2*). Downcore vegetative cell concentrations modeled therein were converted to endospore concentrations at one-degree grid resolution by applying the ratio of endospores to vegetative cells against depth: log endospores/cells = −2.295 + 0.990 log depth. To account for analytical offsets between dPCR-based data and direct cell counts of vegetative cells, as used in (*2*), we previously applied an empirically obtained conversion factor (*30*). Numerical integration of these curves and summation of data yield a global estimate for endospores and vegetative cells.

Details on the analytical methods, the calculation of vegetative cell and endospore abundance and biomass, and the different adjustments made are provided in section S2. All log values presented in the text and figures refer to the logarithm to the base 10. Regressions, linear and segmented linear, and correlation calculations were performed using the Statistics feature of SigmaPlot v.11.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/5/2/eaav1024/DC1

Section S1. Assessment of potential preservation effects of DPA

Section S2. Supplementary materials and methods

Fig. S1. Relationship between endospore and TOC concentrations in the analyzed samples.

Fig. S2. Location of sites analyzed in the present study.

Fig. S3. Endospore concentration over depth in four selected sites.

Fig. S4. Linear regression of the parameters log(*b*) and *m* with POC concentration.

Table S1. Overview of sites analyzed for endospore abundance.

Table S2. Parameters tested for their correlation to endospore abundance at 1 mbsf (*b*) and decrease rate with depth (*m*).

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