Aerobically respiring prokaryotic strains exhibit a broader temperature–pH–salinity space for cell division than anaerobically respiring and fermentative strains

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1. Introduction

The biosphere can be defined as the physical and chemical parameter space that sustains all biological processes on the Earth. Determining how different stress parameters limit microbial multiplication within extreme habitats, and the mechanisms by which life adapts to biologically hostile environments, is of significance to several fields including ecology, food security, biotechnology and astrobiology [1–5]. Additionally to research into the in situ activities of microbial communities under extreme conditions [6–8], the isolation and physiological characterization of microbial strains has been essential to our knowledge of the physico-chemical limits for life, as it has enabled us to investigate the growth phenotypes of extremophilic taxa in detail. Based on cardinal growth data (minimal, optimal and maximal conditions for cell division), several efforts to characterize the global-scale boundary space for bacterial and archaeal cell division have now been made, using two- and three-dimensional maps of habitability [9–12].

Although mapping the boundaries of the biosphere has advanced our understanding of how diverse parameters (such as temperature, pH, salinity and hydrostatic pressure) can limit cell division both individually and in combination,
several of the mechanisms that control the habitability of extreme environments are yet to be identified. Life within these environments is energetically demanding, and thermodynamic limits to habitability have been proposed [13–22]. For example, the Gibbs free energy (and, consequently, adenosine triphosphate (ATP)) yields generated during dissimilatory metabolism have been suggested to determine the ability of several prokaryotic taxa to multiply within high-salt environments [19]. Adaptation to low-energy conditions has additionally been proposed as a factor that distinguishes between the temperature and the pH preferences of thermophilic bacteria and archaea [23, 24]. Global-scale limits to cell division, however, have not been determined for individual modes of catabolism, particularly with reference to multiple-stress conditions that characterize the majority of extreme environments on the Earth [12, 25, 26]. For instance, as aerobic respiration frequently results in high cellular growth yields [27] and can enable ATP yields up to an order of magnitude higher than those attained anaerobically (with up to 36 ATP molecules for each oxidized glucose molecule), could this type of metabolism enable cell division over a broader range of physical and chemical extremes than other catabolic pathways?

In simple terms, energy-yielding reactions can be divided into those relying on ion-gradient-driven phosphorylation and those based on substrate-level phosphorylation [28]. Aerobic and anaerobic respiration use the former, whereas both types of phosphorylation can be involved in fermentation [29–32]. These categories (and anaerobic respiration in particular) encompass an incredible diversity of metabolic reactions that widely differ in terms of their Gibbs energy yields [33–35]. For example, nitrate reduction can result in energy yields close to those obtained by aerobic respiration (with redox potentials ranging up to +0.75 V for reaction pairs involving nitrate and +0.82 V for O2/H2O), while energy yields from the reduction of organic compounds can be nearly an order of magnitude lower [36]. Although the redox potentials of certain anaerobic respiratory pathways are similar to those involving oxygen, both ATP yields and cellular growth yields are relatively low for the former, partially because of differences in the efficiency of aerobic and anaerobic electron transport systems [36]. Furthermore, energy yields from fermentation are frequently lower than those attained through aerobic and anaerobic respiration [30–32, 37].

Environmental conditions (including temperature, pH and salinity, as well as substrate availability and geochemical composition) can significantly influence the activity rates and energy yields of microbially mediated reactions [34, 37–40], which hinders our ability to predict global-scale growth limits for different modes of catabolism, especially when multiple-stress interactions are considered. Even so, under conventional reference conditions (i.e. 25°C, neutral pH, an ionic strength of 0.1 M and atmospheric pressure; see [39]), both the volumes of three-dimensional windows for cell division [12] and group-specific tolerance ranges for a given stress parameter can be expected to differ in the order aerobic respiration > anaerobic respiration > fermentation. Here, we use growth data published for 241 prokaryotic strains to systematically compare the temperature, pH and salinity (NaCl) values for cell division between catabolic groups (based on the terminal electron acceptor used during growth range experiments). While the groups are found to differ with reference to their temperature–pH–salinity space for cell division, particularly between aerobic and anaerobic strains, these patterns are shown to be in limited agreement with simple predictions of energy yields under conventional conditions. To facilitate investigation of the mechanisms underpinning our results, we highlight key areas of research into the ecology, physiology and energetics of extremophilic microorganisms, and the habitability of extreme environments.

### 2. Experimental methods

#### 2.1. Data retrieval and construction of three-dimensional maps

Previously published cardinal growth data were collected for 241 prokaryotic extremophilic or extremotolerant strains (132 genera and two unclassified isolates), consisting of minimal, optimal and maximal conditions supporting cell division with reference to temperature (°C), pH and salinity (% w/v of NaCl) (electronic supplementary material, table S1). Depending on the experimental conditions used for determining cardinal values for cell division, each strain was allocated to a catabolic group consisting of either taxa with O2 as the terminal electron acceptor (aerobic respiration), other inorganic substrates (Fe(III), S0, S compounds or NO3−) as terminal electron acceptors (anaerobic respiration using inorganic electron acceptors) or organic compounds as the electron acceptor (anaerobic respiration using organic electron acceptors and fermentation). A stricter classification of anaerobically respiring versus fermentative strains was precluded because of challenges associated with the extraction of data from previously published articles. Regardless, as Gibbs energy yields from using organic compounds as terminal electron acceptors during anaerobic respiration are often more comparable with

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**Table 1.** Proportions of fully observational, mean-based, median-based and forced cardinal growth values employed for data analysis (see §2.1 for details).

<table>
<thead>
<tr>
<th>catabolic group</th>
<th>total no. values</th>
<th>fully observational</th>
<th>mean-based</th>
<th>median-based</th>
<th>median-based (forced)</th>
</tr>
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<tbody>
<tr>
<td>aerobic respiration</td>
<td>954</td>
<td>84</td>
<td>2</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>anaerobic respiration (inorganic terminal electron acceptors)</td>
<td>648</td>
<td>82</td>
<td>3</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>anaerobic respiration and fermentation (organic terminal electron acceptors)</td>
<td>630</td>
<td>84</td>
<td>2</td>
<td>13</td>
<td>1</td>
</tr>
</tbody>
</table>
those obtained via fermentation than to other respiratory pathways, the approach employed is likely to provide a meaningful distinction between the three groups examined in this work. A lack of sufficient data prevented the inclusion of other types of anaerobic catabolism (e.g. manganese, arsenate and selenite reduction and methanogenesis [19]) in our study. Strains for which cardinal growth values were determined for several electron acceptors were allocated to more than a

Figure 1. Three-dimensional boundary spaces for the multiplication of aerobically respiring, anaerobically respiring and fermentative prokaryotic strains, determined as a function of temperature (°C), pH and NaCl concentration (% w/v). (a) Total dataset; (b) strains with O₂ as the terminal electron acceptor (aerobic respiration); (c) strains with inorganic substrates other than O₂ as the terminal electron acceptor (anaerobic respiration); and (d) strains with organic compounds as the terminal electron acceptor (anaerobic respiration and fermentation). Views of the boundary spaces are provided from three arbitrarily selected angles for each group. The habitable spaces were estimated using cardinal growth data (see §2.1 for details). A total of 241 strains were used (electronic supplementary material, table S1), with each polyhedron corresponding to an individual strain.
and (as functions of temperature (°C), pH and NaCl concentration (% w/v). Limits for cell division are shown for (a) temperature and pH, (b) temperature and salinity, and (c) pH and salinity. The data are split into three catabolic groups (AR, aerobic respiration; ANR (inorg. e\textsuperscript{−}), anaerobic respiration using inorganic terminal electron acceptors; ANR + F (org. e\textsuperscript{−}), anaerobic respiration using organic terminal electron acceptors, and fermentation). Boundaries for overlapping groups are indicated by outlines. The habitable spaces were estimated using cardinal growth data (see §2.1 for details). A total of 241 strains were used, with a full list of strains available in the electronic supplementary material, table S1.

Figure 2. Two-dimensional boundary spaces for the multiplication of aerobically respiring, anaerobically respiring and fermentative prokaryotic strains, determined as functions of temperature (°C), pH and NaCl concentration (% w/v). Limits for cell division are shown for (a) temperature and pH, (b) temperature and salinity, and (c) pH and salinity. The data are split into three catabolic groups (AR, aerobic respiration; ANR (inorg. e\textsuperscript{−}), anaerobic respiration using inorganic terminal electron acceptors; ANR + F (org. e\textsuperscript{−}), anaerobic respiration using organic terminal electron acceptors, and fermentation). Boundaries for overlapping groups are indicated by outlines. The habitable spaces were estimated using cardinal growth data (see §2.1 for details). A total of 241 strains were used, with a full list of strains available in the electronic supplementary material, table S1.

Figure 3. Canonical analysis of principal coordinates (CAP) ordination of aerobically respiring, anaerobically respiring and fermentative prokaryotic strains. The data are split into three catabolic groups (AR, aerobic respiration; ANR (inorg. e\textsuperscript{−}), anaerobic respiration using inorganic terminal electron acceptors; ANR + F (org. e\textsuperscript{−}), anaerobic respiration using organic terminal electron acceptors, and fermentation). The ordination was derived from a Euclidean similarity matrix calculated from normalized cardinal growth data (n = 29 for each grouping). Randomly selected strains were used for the ordination (see §2.2 for details). (Online version in colour.)

2.2. Data analysis and statistics
Volumes of the three-dimensional maps corresponding to specific catabolic groups (% of the total volume estimated for all strains) were calculated by Monte Carlo integration, using 10\textsuperscript{3} integration steps. Using randomly selected strains for which data for all growth variables were available (n = 29 for each group), a Euclidean similarity matrix of normalized cardinal growth values was constructed using the PRIMER statistical package (v. 6.1.13) with the PERMANOVA+ add-on (v. 1.0.3) [41,42]. Randomly selected strains were used in order to obtain a balanced design for statistical analysis. Following unconstrained and constrained ordinations of these data (non-metric multi-dimensional scaling and canonical analysis of principal coordinates, respectively [43]), a one-way permutation analysis of variance (PERMANOVA) was performed with ‘mode of catabolism’ as the factor (Type III sums of squares, 9999 unrestricted permutations of the raw data) [44]. PERMANOVA is conceptually related to traditional analyses of variance (ANOVA), and is based on a non-parametric and distribution-free approach that enables reliable comparisons of multivariate data between sample groups. Post hoc pairwise comparisons were performed using the same PERMANOVA settings. The SIMPER routine was used to calculate the relative contributions (%) of each cardinal growth variable to differences between catabolic groups. Variation in within-group
dispersion was assessed by a test for the homogeneity of multivariate dispersions (PERMDISP using distances from centroids and 9999 permutations) [45]. The PERMDISP routine is used as an accompaniment to PERMANOVA and provides information on whether significant differences between sample groups are due to differences in location (in multivariate space), dispersion (within-group variability) or both.

Strain-specific temperature, pH and salinity (% w/v of NaCl) ranges were calculated as the difference between maximal and minimal values for cell division. Strains for which these values were unavailable were omitted from statistical analysis. One-way ANOVA and post hoc Tukey’s honestly significant difference (HSD) tests for comparing ranges for cell division were performed with ‘mode of catabolism’ as the factor, using the R software package (v. 3.1.0) [46]. The tests were conducted using balanced designs based on the lowest common number of values for a given environmental parameter (n = 67 for temperature; n = 66 for pH; n = 44 for salinity), with values randomly selected from each group where necessary. The data for comparing salinity ranges for cell division were square-root-transformed to achieve normality.

3. Results

3.1. Three-dimensional volumes and limits of the parameter space for cell division

To determine the impacts of temperature, pH and salinity on the window for cell division between aerobically and anaerobically metabolizing prokaryotic isolates, cardinal growth data were employed to construct three-dimensional approximations of the habitable space on the Earth (Experimental methods and electronic supplementary material, table S1). Aerobically respiring strains occupied 90% of the total boundary space (figure 1a,b). Taxa using inorganic electron acceptors other than oxygen and those using organic electron acceptors occupied 15% and 28% of the boundary space, respectively, with considerable overlap between catabolic groups (figures 1 and 2). Despite this overlap, strains from the three groups for which data were available for all growth variables (Experimental methods) differed significantly with reference to their temperature, pH and salinity preferences (one-way PERMANOVA: pseudo-$F_{2,84} = 10.132, p < 0.001, 9930$ unique permutations) (figures 2 and 3; electronic supplementary material, figure S1). Each group occupied a unique position within the total parameter space for cell division (figures 2 and 3; table 2), with temperature, pH and salinity exhibiting distinct contributions (%) to the observed differences (electronic supplementary material, table S2). Multivariate dispersions varied between groups (PERMDISP: $F_{2,84} = 11.145, p < 0.001$), with the aerobic respiration group exhibiting a higher degree of dispersion than other modes of catabolism (figure 3; electronic supplementary material, table S3). No differences in multivariate dispersion were detected between the two anaerobic groups (electronic supplementary material, table S3).

In support of these data, we next performed a comparison of growth minima, optima and maxima between the three catabolic groups. The mean temperature minimum for cell division by aerobically respiring strains (x̄ = 22 ± 2°C s.e.; n = 101) was approximately twofold lower than the mean temperature minima observed for other modes of catabolism (figure 4; see electronic supplementary material, figure S2 for medians). The lowest minimum temperature within the aerobic respiration group (−15°C) corresponded to the bacterium Planococcus halocryophilus [47,48], constituting the lowest temperature within the entire dataset (figures 1 and 2; table 3). By contrast, strains employing organic terminal electron acceptors displayed the highest mean temperature maximum for cell division (x̄ = 77 ± 3°C s.e.; n = 67) (figure 4; also see electronic supplementary material, figure S2), although the highest overall temperature (121°C) was attributed to strains using inorganic electron acceptors other than oxygen (figures 1 and 2; table 3). While the most acidic and alkaline pH values for cell division (0 and 14, respectively) were represented by aerobically respiring isolates, the other two catabolic groups also exhibited cell division under extremes of pH (minimum of 1 and maximum of 11.1; figures 1 and 2; table 3). Moreover, the mean pH minima, optima and maxima for cell division were relatively similar between all three catabolic groups, with the aerobic respiration group exhibiting the highest degree of variability in comparison with other groups (figure 4; electronic supplementary material, figure S2). The highest mean salinity maximum for cell division (x̄ = 17 ± 1 s.e. w/v of NaCl; n = 74) corresponded to aerobically respiring strains (figure 4). Although the highest NaCl concentration within the dataset (36% w/v) also corresponded to this group, this value was only 2% higher than the upper salinity limit for cell division by strains using organic terminal electron acceptors (figures 1 and 2; table 3).

3.2. Temperature, pH and salinity ranges for cellular multiplication

Strain-specific temperature ranges for cell division differed between the catabolic groups (one-way ANOVA: $F_{2,195} = 11.89, p < 0.001$), with a Tukey’s HSD test indicating that temperature ranges for the anaerobic respiration group were lower (p < 0.05) than those observed for the other groups (figure 5a). Temperature ranges for cell division did not differ significantly between aerobically respiring strains and strains using organic terminal electron acceptors (figure 5a). Similar results based on one-way ANOVA ($F_{2,195} = 21.20, p < 0.001$) and Tukey’s HSD comparisons were observed with reference to these data.
Growth values for aerobically respiring, anaerobically respiring and fermentative prokaryotic isolates. Data are shown for minimal (‘min.’), optimal (‘opt.’) and maximal (‘max.’) conditions for cell division, corresponding to (a) temperature (°C), (b) pH and (c) salinity (% w/v of NaCl). The data are split into three catabolic groups (AR, aerobic respiration; ANR (inorg. e–), anaerobic respiration using inorganic terminal electron acceptors; ANR + F (org. e–), anaerobic respiration using organic terminal electron acceptors, and fermentation). The growth values are presented as means ± s.e., with frequencies of strains indicated within or above the bars.

4. Discussion

Energetic factors have been proposed as a determinant of habitability within extreme environments [15–17,19–23]. Our understanding of this topic, however, has been limited by a shortage of research into the ability of different catabolic groups to tolerate physico-chemical extremes [12,25]. Here, we analysed 2232 cardinal growth values corresponding to 241 prokaryotic isolates (electronic supplementary material, table S1) to compare the temperature, pH and salinity values for cellular multiplication between aerobically respiring taxa (oxygen as the terminal electron acceptor), strains using inorganic electron acceptors other than oxygen during respiration, and taxa employing organic electron acceptors (either fermentation or anaerobic respiration). Aerobically respiring strains covered a higher relative volume of the total temperature–pH–salinity space for cell division than either anaerobic group (figures 1 and 2). Each group occupied a unique position within the overall three-dimensional space, with differences between the aerobic respiration group and the other modes of catabolism being partially attributable to the high degree of multivariate dispersion exhibited by this group (figure 3; electronic supplementary material, figure S3). The significant differences observed between all three groups demonstrate that these categories, despite the broad spectrum of catabolic pathways they encompass, are meaningful from the viewpoint of understanding how different types of metabolism map onto the global boundary space for life.

While the difference observed between aerobically and anaerobically metabolizing strains was in agreement with simplified energetic predictions (see Introduction section), the volume for cell division by isolates using organic compounds as terminal electron acceptors exceeded that of strains using inorganic electron acceptors other than oxygen (figures 1 and 2). This finding, combined with a comparison of cardinal growth values (figure 4 and table 3) and ranges (§S3.2; figure 5; electronic supplementary material, figure S3), strongly suggests that predictions of Gibbs energy yields under a limited set of reference conditions (25°C, neutral pH, an ionic strength of 0.1 M and atmospheric pressure) are insufficient for predicting limits for prokaryotic cell division within multiple-stress environments. This conclusion is in agreement with previously published research which has shown that Gibbs energy yields depend on several physico-chemical factors (including temperature, pH, salinity, pressure and the surrounding chemical composition), as well as substrate availability [15,34,38,39]. For example, Amend & Shock [39] calculated Gibbs free energies for over 300 reactions as a function of temperature and concluded that accounting for extremes of this parameter (as well as other conditions beyond the typical bioenergetic reference frame) is essential to understanding the ecology of thermophilic taxa. As our dataset encompassed temperatures ranging from −15 to 121°C, pH values from 0 to 14 and NaCl concentrations of 0–36% (w/v), this study highlights the need for research into microbial bioenergetics across the entire range of conditions.
reported. In particular, this work shows the need to consider interactions between multiple-stress parameters in order to formulate accurate predictions of cellular tolerance to extremes [39]. Crucially, to correctly apply these predictions to natural ecosystems, any calculations of Gibbs energy yields would also need to account for the concentrations of all reactants and products corresponding to a given reaction. Anabolic factors are additionally likely to be important to estimating limits to cell division, and are similarly dependent on environmental conditions. For example, the energetic cost of synthesizing biomass has been found to be lower under anaerobic conditions than in aerobic environments [69,70], which could partially underpin the results reported in our study.

Further to these considerations, several other variables may influence the physico-chemical growth limits of different catabolic modes. For example, experiments using sediment microbial consortia have showed that biological factors (e.g. cellular free-energy efficiency, population viability and mutualism) can exert a greater degree of control over rates of microbial catabolism than the availability of usable energy ([71] and references therein). Given our results, it is possible that such factors can also influence the distribution of diverse modes of catabolism within the global-scale parameter space for life. Indeed, a number of physiological mechanisms could explain the ability of several anaerobically metabolizing taxa to multiply under hypersaline conditions (figures 4c, 5c; electronic supplementary material, figures S2 and S3), as discussed by Oren [13,14,19]. The ‘salt-in’ mechanism of osmoadaptation, for example, provides an energetically efficient alternative to synthesizing compatible solutes, which may enable certain isolates (such as *Haloanaerobium lacusroseus*) to carry out fermentation at extreme salinity (table 3) [13,14,19]. Energy-conserving strategies, which include diverse structural, biochemical and ecological adaptations, can also facilitate cellular maintenance and multiplication under extreme temperatures and pH [21,23,24,72–77]. For example, the lipid structure of the archaeal cell membrane is likely to be adapted for life under nutrient-poor conditions that are common within biologically hostile environments [23,24]. Modifications of the cytoplasmic membrane (along with other adaptations) are additionally known to help sustain pH homeostasis under extremes of pH [72,76].

<table>
<thead>
<tr>
<th>group</th>
<th>parameter</th>
<th>limit</th>
<th>strain</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Planococcus halocryophilus</td>
<td>[47,48]</td>
</tr>
<tr>
<td></td>
<td>max. temp. (°C)</td>
<td>121</td>
<td>strain 121</td>
<td>[49]</td>
</tr>
<tr>
<td></td>
<td>min. pH</td>
<td>0</td>
<td>Acidiplasma aerilicum</td>
<td>[50]</td>
</tr>
<tr>
<td></td>
<td>max. pH</td>
<td>14</td>
<td>Ferrophilus acidarmanus</td>
<td>[51]</td>
</tr>
<tr>
<td></td>
<td>max. NaCl (% w/v)</td>
<td>36</td>
<td>Picrophilus oshimae</td>
<td>[52,53]</td>
</tr>
<tr>
<td>aer. resp.</td>
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<td>as for total dataset</td>
<td></td>
</tr>
<tr>
<td></td>
<td>max. temp. (°C)</td>
<td>104</td>
<td>Pyrobaculum aerophilum</td>
<td>[56]</td>
</tr>
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<td>0</td>
<td>as for total dataset</td>
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<td></td>
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<td>14</td>
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<td></td>
<td>max. NaCl (% w/v)</td>
<td>36</td>
<td>as for total dataset</td>
<td></td>
</tr>
<tr>
<td>anaer. resp. (inorganic e− acceptors)</td>
<td>min. temp. (°C)</td>
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<td>Desulfuromonas stutvbardensis</td>
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</tr>
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<td>Acidianus infernus</td>
<td>[58]</td>
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<td>Acidianus manzaensis</td>
<td>[59]</td>
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<tr>
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<td>Marinilactibacillus psychrotolerans</td>
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<td>Hyperthermus butylicus</td>
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<td></td>
<td>min. pH</td>
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<td>Pyrococcus yayanosii</td>
<td>[65]</td>
</tr>
<tr>
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<td>[66]</td>
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<tr>
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<td></td>
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<td>11.1</td>
<td>Haloanaerobium lacusroseus</td>
<td>[68]</td>
</tr>
</tbody>
</table>

aFor a complete list of strains, see electronic supplementary material, table S1.
While physiological and/or evolutionary factors are likely to have played a key role in determining the position of each catabolic group within the total parameter space for cell division (tables 2 and 3, figures 1–5), it is possible that some of the results reported in our study were influenced by purely physical or chemical processes. The low solubility of oxygen at high temperatures and the presence of reducing conditions within hyperthermophilic environments, for example, could contribute to the tendency of aerobically respiring taxa to exhibit lower temperature preferences compared with anaerobically respiring and fermentative strains (figure 4a) [78]. Although the known temperature window for cell division may expand as a result of further research, ultimately the thermal boundaries for life are set by limits to macromolecular stability and access to liquid water [16,20,79]. A common water-activity limit (approx. 0.61) to cellular multiplication has been proposed for all three domains of life [80]. Improvements in the availability of suitable growth data (e.g., via the targeted isolation and characterization of extremophlic strains) are therefore needed to improve our ability to determine the extent to which such factors affect the known limits for cell division on the Earth (table 3, figures 1 and 2).

Metagenomic analyses have previously demonstrated a strong correlation between environmental conditions and microbial strategies for energy conversion and conservation [81,82]. While such analyses would complement culture-based attempts to advance our knowledge of the distribution of different metabolic pathways within the temperature–pH–salinity space for prokaryotic multiplication on the Earth (figure 1a), they would require an unprecedented sampling effort. Indeed, global metagenome surveys to date have focused on pelagic marine habitats [83–85]. Additionally, attempts to define the habitable parameter space on the Earth have necessarily been restricted to data obtained for isolates [9–12]. As the vast majority of microorganisms are unculturable in the laboratory [86], it remains unclear how representative the taxa employed in our study are of those that define the temperature, pH and salinity limits for cell division within natural environments. Furthermore, as laboratory experiments are typically conducted under nutrient-rich conditions [87] and in the absence of several stresses encountered in the environment (e.g., predation, competition and physico-chemical parameters including extremes of radiation or pressure), results from strain-based investigations are likely to be poorly correlated with in situ patterns of microbial activity and limits to cell division [12,23,88]. Indeed, rates of microbial metabolism are often likely to be lower within natural environments than under laboratory conditions, with important implications for microbial growth and persistence. While aerobically respiring communities can occur within marine sediments to a depth of up to 75 m, for example, both cell numbers and rates of microbial activity are very low within these environments [89].

Regardless of uncertainties associated with strain- and laboratory-based attempts to determine the boundaries of the biosphere [12], the results of this study have broad implications for our knowledge of ecosystem processes within extreme environments, and the global-scale physico-chemical limits for microbial cell division. The data presented herein are consistent with the theory that the evolution of an oxygen-rich atmosphere may have enabled microorganisms to exploit a broader global niche space compared with an early and oxygen-poor Earth [90], but they also highlight the ability of

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**Figure 5.** Growth ranges for aerobically respiring, anaerobically respiring and fermentative prokaryotic isolates. Data are shown for (a) temperature (°C), (b) pH and (c) salinity (% w/v of NaCl). The data are shown as untransformed means ± s.e., with frequencies of strains indicated under the sample labels (AR, aerobic respiration; ANR (inorg. e−), anaerobic respiration using inorganic terminal electron acceptors; ANR + F (org. e−), anaerobic respiration using organic terminal electron acceptors, and fermentation). Different letters above the bars indicate significant differences between individual modes of catabolism (p < 0.05 (Tukey’s HSD test following one-way ANOVAs with balanced designs; see §3.2 for ANOVA results)).
low-ATP-yielding modes of catabolism to support prokaryotic multiplication under conditions that define present-day limits for life. Therefore, our results support the potential for severely energy-limited habitats (e.g. deep subsurface environments) to sustain a surprisingly broad spectrum of microbial activities [15,23,91]. To advance our ability to predict cellular growth limits within extreme environments, there is a need to further integrate investigations of microbial ecology, physiology and energetics, particularly with reference to global-scale processes. Entire metabolic networks, for example, could be considered in order to build a fuller understanding of how energy transduction affects habitability [16]. Moreover, systems approaches (including ‘omics’ methods [92]) are likely to play an important future role in improving our knowledge of the physico-chemical limits for microbial cell division [93]. Such approaches are particularly necessary to advance our understanding of how diverse stress parameters (and physiological adaptations to them) interact, and how these interactions shape the biophysical boundaries for life on the Earth.

5. Conclusion
By systematically comparing minimal, optimal and maximal values for cell division between 241 prokaryotic strains, we have shown that aerobically respiring isolates are characterized by a broader temperature–pH–salinity window for cell division than strains which employ other inorganic substrates or organic compounds as the terminal electron acceptor. We also found that taxa employing organic terminal electron acceptors displayed a broader habitable window in comparison with anaerobically respiring strains that used inorganic substrates as the electron acceptor, which was not anticipated by simple energetic predictions (relative Gibbs energy yields under the conventional reference conditions of 25°C, neutral pH, an ionic strength of 0.1 M and atmospheric pressure). Our findings, therefore, highlight the need for global-scale models of habitability to account for the impacts of environmental conditions (and in particular, interactions between extremes) on cellular energetics [94]. Such models will also require consideration of anaerobic reactions, as well as substrate availability and rates of microbial activity (substrate consumption). Further to these issues, it is possible that one of the factors underpinning our results is the ability of physiological and/or evolutionary adaptations to extremes to enable low-ATP-yielding modes of catabolism (such as fermentation) to operate under severely energy-limited conditions (e.g. [19]). Our ability to successfully predict the habitability of extreme environments on both the Earth and the other planetary bodies, therefore, is reliant on our knowledge of the complete set of biotic and abiotic mechanisms that permit microbial life to persist under multiple-stress conditions.

Although research into the Earth’s habitable window is limited by the availability of suitable cardinal growth (and other strain-specific) data [12], this issue could be alleviated by improving the transparency and accessibility of information for previously described and novel microbial strains. Cardinal growth values for newly described isolates, for example, could be determined for individual and combined stress parameters, with the additional adoption of a standardized format for reporting fundamental growth properties (including a list of possible electron donors and acceptors, and specifically those employed during growth range experiments). To extend the validity of laboratory measurements of microbial growth to natural systems, it would also be necessary to carry out energetic calculations, or report the variables required for doing so. Further to other parameters (such as temperature and pressure), these include concentrations of electron donors and acceptors over time, the ionic strength of the solution and the concentrations of metabolic reactants and products. Experimental and theoretical approaches to address questions such as: ‘Do auto- versus heterotrophic microbial taxa differ in their tolerance of environmental extremes?’ and ‘How do interspecific interactions (including syntrophy) influence the physical and chemical limits for microbial multiplication?’ [71,95,96] could additionally advance our knowledge of the global-scale boundaries for cell division.

Data accessibility. Supplementary figures and tables, including a full list of the strains used in this work, are available in the electronic supplementary material.

Authors’ contributions. J.P.H. and C.S.C. designed the study; all authors assembled data for analysis; L.D. constructed the three-dimensional maps and performed Monte Carlo integrations; L.D. and J.P.H. constructed the two-dimensional maps; J.P.H. performed statistical tests and wrote the initial draft of the manuscript; all authors contributed to and approved the final version of the manuscript.

Competing interests. We declare we have no competing interests.

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References
heterotrophic, thermoacidophilic genus and family comprising archaea capable of growth around pH 0. J. Bacteriol. 177, 7050–7059.


60. Stevens et al. 2015. Is there a common water-activity limit for the three domains of life? ISME J. 9, 1333–1351. (doi:10.1038/ismej.2014.219)


