A distinct growth physiology enhances bacterial growth under rapid nutrient fluctuations

Jen Nguyen1,2, Vicente Fernandez1, Sammy Pontrelli3, Uwe Sauer3, Martin Ackermann4,5 & Roman Stocker1

It has long been known that bacteria coordinate their physiology with their nutrient environment, yet our current understanding offers little intuition for how bacteria respond to the second-to-minute scale fluctuations in nutrient concentration characteristic of many microbial habitats. To investigate the effects of rapid nutrient fluctuations on bacterial growth, we couple custom microfluidics with single-cell microscopy to quantify the growth rate of E. coli experiencing 30 s to 60 min nutrient fluctuations. Compared to steady environments of equal average concentration, fluctuating environments reduce growth rate by up to 50%. However, measured reductions in growth rate are only 38% of the growth loss predicted from single nutrient shifts. This enhancement derives from the distinct growth response of cells grown in environments that fluctuate rather than shift once. We report an unexpected physiology adapted for growth in nutrient fluctuations and implicate nutrient timescale as a critical environmental parameter beyond nutrient identity and concentration.
Our planet is sustained by the metabolic activities of microorganisms. In our gut, microbial communities break down nutrients into forms that we can take up and use; at sea, microbial growth affects the sequestration of carbon in the ocean and its release back into the atmosphere; and in the soil, microbes convert organic molecules into forms that facilitate plant growth. These metabolic activities are often performed under conditions that depart from a steady state. Rather, the quality and quantity of available nutrients often fluctuate rapidly due to microscale spatial heterogeneity, fluid flow, or host eating habits. Many host-associated and free-living microbes experience second- and minute-scale fluctuations in nutrient availability as they swim through resource landscapes that are highly heterogeneous at sub-millimeter scales. Surface-attached microorganisms experience rapid changes in resources through the movement of the liquid around them.

To understand the impacts that microorganisms have on the physiology of their hosts and on global elemental cycles, we have to understand how individual bacteria respond to nutrient fluctuations. However, our understanding of microbial physiology draws heavily on knowledge derived from steady environments or single transitions between steady states. Microbial metabolism and growth under nutrient fluctuations remains a knowledge gap, largely due to the technical challenges of studying cells in highly dynamic environments. Here, we address this gap using single-cell growth experiments in a custom microfluidic device to show that rapid fluctuations substantially diminish growth, but also that bacteria can exhibit a fluctuation-adapted growth physiology that enhances growth under frequent environmental change.

Recent advances in single-cell measurement techniques have laid foundations for considering the implications of second- and minute-scale fluctuations on bacterial growth and physiology. Single-cell measurements of bacterial mass at femtogram resolution have confirmed that individual bacteria add mass exponentially. Experiments enabled by a groundbreaking microfluidic tool, the Mother Machine, revealed that the exponential growth of individual cells is stable over hundreds of generations, indicating that steady-state growth applies not only at the population level but also to individuals. This seminal discovery has catalyzed major progress toward understanding the homeostatic regulation by which bacteria tune their growth and physiology to their environment, resulting, for example, in the Adder model of cell-size control and hypotheses for its underlying mechanisms. This wealth of literature stems from and reinforces a long-standing paradigm that each nutrient environment induces a characteristic steady-state growth rate, in which cells tightly regulate their size, proteome, and biosynthesis rates in response to nutrient availability. The robustness of steady-state cell physiologies has led to growth laws that relate physiological traits, such as RNA–protein ratios, with steady-state growth rate.

The expansive experimental and theoretical characterization of steady-state growth has led to its use as the framework to interpret bacterial physiology and ecology, even in dynamic environments. Currently, our understanding of bacterial responses to changes in the environment derives heavily from characterizations of physiological transitions from one steady state to another. Upon a nutrient shift out of steady state, cells initiate a cascade of responses that depend on the nutrient composition of the new environment and require hours to complete. Specific processes respond over a range of timescales—transcription over seconds, translation over minutes, cell division over hours—and the progression of these physiological changes is reflected in a cell’s growth rate. The dynamics of growth transitions thus provide insight into the strategies employed by bacteria and the ecological challenges under which these strategies have evolved.

Steady-state growth is highly informative when nutrients fluctuate on timescales longer than the timescales required for cells to transition between steady states; however, it is unclear whether it provides an appropriate framework for understanding physiology when nutrients fluctuate on timescales of seconds or minutes. For example, minute-scale alternation in the expression of metabolic pathways associated with different steady states may produce cells with proteins that are not usually co-exRESSED. Proteins expressed during prior exposures to a condition might reduce lag times when that condition returns, yet unnecessary gene expression can also reduce growth rate. Understanding how frequent and repeated shifts in nutrient concentration integrate to affect bacterial growth physiology requires systematic study of single-cell growth under rapid nutrient fluctuations.

In this study, we characterized the rate and dynamics of bacterial growth under fluctuations between two fixed nutrient concentrations on timescales of seconds to minutes. Using a custom microfluidic device that precisely controls nutrient concentration over time, we quantified the growth dynamics of thousands of individual Escherichia coli cells exposed to identical, periodic nutrient fluctuations with periods as short as 30 s. We found that rapid nutrient fluctuations reduce growth rate by up to 50% when compared to a steady nutrient condition delivering the equal average concentration. However, the measured loss is considerably smaller than the growth loss expected from a null model based on measured growth responses to single shifts in nutrient concentration. Here, we provide the first evidence of a fluctuation-adapted growth physiology that alleviates growth loss in fluctuating nutrient environments and implicate continued temporal variability as a fundamental parameter for understanding bacterial physiology in dynamic habitats.

Results and discussion
Exposing single bacteria to precisely controlled, rapid nutrient fluctuations. To determine how rapid nutrient fluctuations affect bacterial growth, we engineered a microfluidic device to rapidly switch between the delivery of two different nutrient concentrations while simultaneously imaging individual bacteria with time-lapse phase-contrast microscopy. We grew surface-attached E. coli under nutrient oscillations with periods of 30 s, 5 min, 15 min, or 60 min. Each experiment began by flowing cells from a growing batch culture into the device and allowing them to adhere onto its glass surface prior to initiating nutrient fluctuations (Methods and Supplementary Fig. 2). Switches between the two nutrient concentrations occurred while maintaining a constant flow rate and each switch was completed in <3 s (Supplementary Fig. 3). The resulting nutrient signal was reliably experienced by the cells as a square wave of equal time (half the period) in each concentration (Fig. 1b), with sharp transitions between concentrations (Fig. 1c).

Neither nutrient depletion nor metabolite accumulation altered the composition of the nutrient media experienced by the cells, due to the high flow rates and channel depth (60 µm) used (Supplementary Fig. 4 and Supplementary Table 1). Usually, one of the two cells emerging from each cell division event was transported away by the flow (Fig. 1d), allowing us to acquire time series of thousands of individual cells (4000–20,000) for each experiment. We confirmed that growth rate was independent of a cell’s position along the 10-mm-long region imaged within the microchannel (Supplementary Fig. 4) and that cells within the same condition therefore experienced identical nutrient signals over time.
that varying nutrient concentration reproduced key relationships between growth rate, cell size, and division time (Supplementary Fig. 5c, d) previously established by varying nutrient source (e.g., glucose vs. tryptic soy broth)\(^\text{17}\), suggesting that variations in growth rate due to changes in nutrient concentration and nutrient source are physiologically similar. Importantly, the \(C_{\text{ave}}\) control provided cells with average and total nutrient identical to that in the fluctuating environments. Together, these steady controls enabled us to distinguish the effects of fluctuation timescale from those of nutrient concentration by providing reference growth rates in steady low, average, and high nutrient concentrations.

**Growth rate rapidly responds to nutrient fluctuations.** This microfluidic system allowed us to measure the growth rate of individual cells in steady and fluctuating nutrient concentrations with high precision and temporal resolution. Growth rate, defined here as the rate at which cell volume doubles, was quantified from phase-contrast images of single cells acquired approximately every 2 min (see “Methods”). For each cell imaged, we extracted the length and width using image analysis and quantified cell volume, \(V(t)\), by approximating the cell as a cylinder with hemispherical caps\(^\text{18, 28}\). Using the resulting time series of cell volume (Fig. 2a), we computed the instantaneous growth rate for each single cell, \(\mu(t)\), from \(V(t + \Delta t) = V(t) \times 2^{\Delta t/\Delta t}\) (see “Methods”). We focused on single-cell volumetric growth rather than cell replication or division time because biomass production has been shown to respond within minutes of a nutrient shift, whereas cell division responds more slowly (within an hour)\(^\text{23}\).

The growth rate in the steady controls stabilized within 3 h of the start of the experiments (Supplementary Fig. 5). Thus, the steadystate growth rates (\(G_{\text{high}}, G_{\text{ave}}, \text{ and } G_{\text{low}}\)) from steady \(C_{\text{high}}, C_{\text{ave}}, \text{ and } C_{\text{low}}\) were computed by averaging all single-cell growth rates, \(\mu\), measured after 3 h within each respective condition (Fig. 2b). We confirmed with metabolomic profiling that the different steady-state growth rates between the three steady conditions resulted from changes in nutrient uptake rates, rather than changes in preferred metabolites (i.e., serine is likely the preferred metabolite across all conditions) (“Methods” and Supplementary Fig. 6).

Growth rate in the fluctuating environment changed rapidly in response to changes in nutrient concentration. Because instantaneous growth rate dynamics from single cells were noisy (Supplementary Fig. 7), we averaged the single-cell growth rates at each time point to visualize the dynamics of time-averaged instantaneous growth rates, which displayed strong and sharp fluctuations, changing more than two-fold within minutes (Fig. 2b). Instantaneous growth rate fluctuated with the immediate nutrient concentration, such that higher growth rates were observed in the high-nutrient phase and lower growth rates in the low-nutrient phase (Fig. 2b). While cell divisions occurred more frequently during phases of high nutrient, we confirmed that the fluctuations in growth rate reflected responses in single-cell volume, rather than cell division responses (Supplementary Fig. 8). When averaging the instantaneous growth rates from each phase of the nutrient signal, periodic changes were observed when nutrients fluctuated with a period of 5, 15, or 60 min (Fig. 2c), indicating that growth rate responded to nutrient shifts in <2.5 min. Changes in growth rate that slightly precede changes in the nutrient signal are not an anticipatory response, but rather are caused by limits in the time resolution of our measurements (Supplementary Fig. 9a). Similarly, fluctuations in growth rate were not resolvable with 30 s fluctuations, owing to the temporal resolution of image acquisition (2 min) (see Image acquisition in “Methods”). Nevertheless, these results establish that rapid

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**Fig. 1** The microfluidic signal generator (MSG) creates automated, precise high-frequency fluctuations in nutrient concentration while enabling single-cell microscopy. a The two channel configurations: the MSG switches between two media (top) and the straight channels each steadily deliver a single medium (bottom). Each experiment contains four parallel channels: one MSG and three straight. The two MSG channels displayed here schematically represent the flow conditions that deliver either \(C_{\text{low}}\) or \(C_{\text{high}}\) to the cells. The upstream portion of the MSG switches the nutrient media delivered to cells via automated control over the pressure differences driving each medium while maintaining a constant flow rate into the device. The top MSG delivers \(C_{\text{high}}\) (red) to cells by pressurizing the red inlet higher than the \(C_{\text{low}}\) (purple) inlet. The wider downstream section fits over ten imaging fields of view \(\times 60\) magnification. b Bacteria were exposed to fluctuating signals in the form of even oscillations between a low and a high LB concentration (\(C_{\text{low}}\) and \(C_{\text{high}}\)), with periods, \(T\), between 30 s and 60 min. Three control environments, \(C_{\text{low}}, C_{\text{ave}}\), and \(C_{\text{high}}\), were run simultaneously with each fluctuating environment. c Fluorescence intensity, from fluorescein added to one of the media, illustrates the signal received at the cell imaging region over multiple oscillations (\(T = 30\) s). Transitions between media are completed in <3 s. d Individual \(E.\ coli\) cells growing within the MSG. Cells were imaged at 117 s intervals; timestamps of selected images are displayed in minutes. Cells divide between \(t_2\) and \(t_3\) (orange) and between \(t_4\) and \(t_5\) (blue), and can be seen elongating between other frames. One of the two cells emerging from division is swept away with the flow. Images were cropped from one steady nutrient experiment and are representative of the growth behaviors observed in all experiments described in this paper.
fluctuations in nutrient concentration lead to minute-scale fluctuations in instantaneous growth rate. We next asked how these nutrient fluctuations—and the resulting fluctuations in instantaneous growth rate—impact the mean rate at which individual cells grow.

Rapid nutrient fluctuations reduce growth rate relative to steady conditions. The mean growth rate in fluctuating environments, \( G_{\text{fluc}} \), was consistently lower than the growth rate in the steady average conditions, \( G_{\text{ave}} \). For each fluctuation timescale, we computed \( G_{\text{fluc}} \) as the average of all instantaneous growth rates for all cells measured from 3 h to the end of the experiment. For nutrient fluctuations on 30 s, 5 min, 15 min, and 60 min periods, this measure yielded \( G_{\text{fluc}} \) values of 1.93 ± 0.16, 1.53 ± 0.20, 1.15 ± 0.28, and 1.15 ± 0.13 h⁻¹, respectively (mean ± standard deviation; \( n = 3-4 \) replicate experiments per condition, each with at least 1842 cells; Fig. 3a and Supplementary Table 2). The corresponding value of \( G_{\text{ave}} \) was 2.31 ± 0.18 h⁻¹ (\( n = 13 \) replicate experiments; Supplementary Table 2). Accordingly, \( G_{\text{fluc}} \) was lower than \( G_{\text{ave}} \) by 16.5-50.2% of \( G_{\text{ave}} \).

This reduction in mean growth rate has strong implications for bacterial population dynamics. For example, for each initial biomass of \( M_0 = 1 \mu m^3 \), the measured \( G_{\text{fluc}} \) from 30 s fluctuations (1.93 ± 0.16 h⁻¹) and \( G_{\text{ave}} \) (2.31 ± 0.18 h⁻¹) correspond to a daily (\( t = 1 \) day) produced biomass of \( 9 \times 10^{13} \) and \( 5 \times 10^{16} \mu m^3 \), respectively (Supplementary Table 3). In other words, two cells of equal initial volume, both growing exponentially \( (M(t) = M_0 \cdot 2^{t/t}) \) with one at rate \( G_{\text{fluc}} \) and one at \( G_{\text{ave}} \) will differ in biomass production by >500-fold in a single day. Thus, by affecting growth rate, the timing of nutrient availability is an important parameter in the growth and productivity of bacterial populations.

Why do nutrient fluctuations reduce growth rate relative to the steady-state growth rate, \( G_{\text{ave}} \)? A simple mathematical model, relevant to the concave Monod curve, illustrates a physiological implication of fluctuating nutrients. In the Monod curve, steady-
state growth rate increases less than linearly with nutrient concentration (Fig. 3b). This steady-state function is an example of Jensen’s inequality, which states that for a concave function, the mean of the function (i.e., \( G_J = (G_{low} + G_{high})/2 \)) is smaller than the mean of the function (i.e., \( G_{ave} \)). This inequality predicts that fluctuations between \( G_{high} \) and \( G_{low} \) would result in a growth rate, \( G_J \), lower than \( G_{ave} \) (Fig. 3b). Still, Jensen’s inequality does not explain the observed growth reductions. The growth rates from fluctuating environments, \( G_{fluc} \), were lower than \( G_J \) for all fluctuation periods except 30 s (Fig. 3c). This difference is consistent with the unrealistic scenario represented by Jensen’s inequality, which considers a cell that fluctuates between growth at two steady states, \( G_{low} \) and \( G_{high} \) (1.07 ± 0.23 and 2.86 ± 0.14 h\(^{-1} \), respectively). In reality, the magnitude by which \( G_{fluc} \) is lower than \( G_J \) depends on the dynamics by which single-cell growth rate responds to fluctuations in nutrient concentration.

We hypothesized that the reduction in growth rate results from the time required for cells to adopt the steady-state physiology characteristic of the current nutrient condition following each fluctuation. The prevailing paradigm for growth transitions presumes that cells initiate a physiological transition to the immediate nutrient environment, regardless of the nutrient timescale. In response to a single shift in nutrient concentration (e.g., from \( C_{low} \) to \( C_{high} \)), cells grow at rates lower than steady-state \( G_{high} \) for several hours until the physiological transition is complete (Fig. 4a). This hours-scale transition in growth rate is a characteristic response to environments in which the nutrient condition shifts only once. When nutrient fluctuations occur on timescales longer than this hours-scale transition in growth rate, predicting growth dynamics from such single-shift responses is relatively straightforward. When nutrient fluctuations occur on timescales faster than the time required to physiologically transition between steady states, the paradigm predicts that cells should never stabilize in growth rate and that cells continuously grow at rates lower than steady state, causing \( G_{fluc} \) to be lower than \( G_J \).

**Growth rate responses differ between repeated fluctuations and single nutrient shifts.** To determine whether the timescale of physiological transitions could explain the reduction in \( G_{fluc} \), we compared the growth rate dynamics between cells exposed to fluctuations and cells exposed to a single up- or downshift in nutrient concentration after having reached steady state prior to the shift. In single-upshift experiments, cells growing steadily at \( G_{low} \) were switched to \( G_{high} \), while in single-downshift experiments, cells growing steadily at \( G_{high} \) were switched to \( G_{low} \). These single-shift experiments enabled us to quantify the time required for cells to physiologically transition between the two steady states, as well as the dynamics of growth rate across the transition (Fig. 4a). In single-upshift experiments, the growth rate gradually increased from \( G_{low} \) until reaching steady-state \( G_{high} \) after 2–3 h. In single-downshift experiments, the growth rate dropped sharply from \( G_{high} \) down to 10% of \( G_{low} \), then gradually increased until reaching steady-state \( G_{low} \) after 5 h (Fig. 4a).

In contrast to cells exposed to single shifts in nutrient concentration, cells grown in fluctuations stabilized at growth rates lower than steady state. We observed this stabilization of growth rate in fluctuating environments with 15 and 60 min periods, which were better resolved with our 2-min imaging interval (Fig. 4b). Growth rate under these fluctuations stabilized at 66% of \( G_{high} \) after each upshift and at 63–68% of \( G_{low} \) after each downshift (Fig. 4c, d and Supplementary Table 4) within minutes: 3.8 ± 0.0 or 3.3 ± 1.4 min after each upshift and 2.2 ± 1.9 min or 15.0 ± 7.6 min after each downshift (\( T = 15 \) or 60 min, respectively) (Fig. 4e). The minute-scale stabilization of growth rate observed from fluctuating conditions was in stark contrast to the hours-long timescale observed from single-shift conditions (116.3 ± 12.4 min after a single upshift and at least 297.5 min after...
Growth rate responses differ between repeated fluctuations and single nutrient shifts. a Average growth rate of single cells over time in four conditions: a single shift in nutrient concentration (occurring at 3 h, after cells had reached steady-state growth in the initial condition), steady $C_{\text{low}}$, steady $C_{\text{ave}}$ and steady $C_{\text{high}}$. On the left, a single nutrient upshift (shift from $C_{\text{low}}$ to $C_{\text{high}}$) and on the right, a single nutrient downshift (from $C_{\text{high}}$ to $C_{\text{low}}$). After each shift, the growth rate gradually reaches steady-state growth in the post-shift condition. The growth rates in $C_{\text{low}}$ before the upshift and in the steady $C_{\text{low}}$ condition are both within the range of measured steady-state $G_{\text{low}}$ (Supplementary Tables 2 and 7). Data are from one representative experiment. The shaded region marks the portion of single-shift data plotted in (b), which does not include the full progression to steady-state growth after a single up- or downshift. b Growth rate of single cells in fluctuating nutrient conditions stabilizes more rapidly and at a lower value when compared to the growth rate dynamics of cells experiencing a single shift. Data were aligned such that the nutrient shift in all conditions occurs at $t = 0$. Post-shift data in fluctuating environments are plotted up until the next shift occurs. Shaded error bars denote the standard deviation of the mean among replicate experiments ($n = 3$–$4$ for fluctuating conditions; $n = 2$ for single-shift conditions). c Growth rate is considered stabilized once the slope of the growth signal within a shrinking window reaches zero. Stabilization time is defined as the time between the nutrient shift and the time at which the growth rate is stabilized. d The growth rate of fluctuation-grown cells stabilized at rates lower than steady-state $G_{\text{high}}$ or $G_{\text{low}}$. Cells experiencing 15 and 60 min fluctuations stabilized at $1.86 \pm 0.47$ and $1.86 \pm 0.13$ h$^{-1}$, respectively, after an upshift and at $0.65 \pm 0.22$ and $0.60 \pm 0.10$ h$^{-1}$ after a downshift. e Cells grown in fluctuations stabilize in growth rate within 3.8 ± 0.0 ($T = 15$ min) or 3.3 ± 1.4 min ($T = 60$ min) of each upshift and within 2.2 ± 1.9 ($T = 15$ min) or 15.0 ± 7.6 min ($T = 60$ min) of each downshift ($n = 3$–4). Cells grown in steady environments stabilize hours after a single shift, 116.3 ± 12.4 min in the case of upshifts ($n = 2$) and at least 297.5 min after a downshift (one of two replicates stabilized after 5 h). f The initial change in growth rate in the minutes following a nutrient shift differed between cells experiencing fluctuations and cells experiencing only a single shift. Over the first 7.5 min after each upshift, cells experiencing fluctuations increased in growth rate relative to their pre-shift growth rate ($t = 0$) by $138.3 \pm 20.6\%$ (15 min) or $158.7 \pm 48.4\%$ (60 min), compared to a 12.0 ± 14.0% increase in the single-shift case. Over the 7.5 min after each downshift, cells experiencing fluctuations decreased in growth rate by $57.8 \pm 3.7\%$ (15 min) or $80.4 \pm 2.6\%$ (60 min), compared to a 90.9 ± 2.5% decrease in growth rate in cells after a single shift. Error bars denote the standard deviation of the mean of $n = 3$ or 4 biologically independent experimental replicates (none displayed for $n < 3$). Overlaid data points represent measurements from each of $n$ replicates.
The full period (under steady C growth physiology period transition from the dynamics observed in response to a single shift to those observed in fluctuating environments (Fig. 4b). The growth rate dynamics of the successive periods, including the 60 min preceding the first nutrient upshift (period 0), are overlaid to show the stabilization of the growth rate signal. Shaded error bars denote the standard deviation of the mean among replicate experiments (n = 3). b Stabilization of growth rate dynamics with successive nutrient periods. The mean growth rate following each downshift (half-period in C low, t low), each upshift (half-period in C high, t high) and across the full period (T) adjusts and stabilizes by the third period. Error bars denote the standard error of the mean (n = 3) and are smaller than points when not visible.

Fig. 5 Rapid nutrient fluctuations induce the transition to a fluctuation-adapted growth physiology. a After at least 3.5 h of growth in steady C low, cells experience the onset of nutrient fluctuations (T = 60 min) between C low and C high. The growth rate dynamics in response to each successive nutrient period transition from the dynamics observed in response to a single shift to those observed in fluctuating environments (Fig. 4b). The growth rate dynamics of the successive periods, including the 60 min preceding the first nutrient upshift (period 0), are overlaid to show the stabilization of the growth rate signal. Shaded error bars denote the standard deviation of the mean among replicate experiments (n = 3). b Stabilization of growth rate dynamics with successive nutrient periods. The mean growth rate following each downshift (half-period in C low, t low), each upshift (half-period in C high, t high) and across the full period (T) adjusts and stabilizes by the third period. Error bars denote the standard error of the mean (n = 3) and are smaller than points when not visible.

A single downshift (Fig. 4e) and is more consistent with a stable growth physiology, i.e., a single cellular composition that grows at different rates due to changes in metabolite flux (Fig. 4f), than changes in gene expression.

These observations show that the effect of rapid nutrient fluctuations on growth cannot be understood by reference to a sequence of single up- and downshifts. Instead, the minute-scale stabilization of growth rate and distinct values of growth rate (once stabilized) suggests that cells grown under fluctuations have a different growth physiology from cells grown under steady nutrient conditions. While we measured different stabilization times between the two fluctuating timescales after downshifts, the overall growth rate dynamics observed from both conditions (T = 15 and 60 min) were more comparable with each other than with the single downshift condition. Growth rate dropped sharply after a shift to C low in fluctuating and single-shift conditions (Fig. 4b), yet growth rate quickly increased in both fluctuating conditions while no such rebound occurred after a single shift (Fig. 4b).

That cells grown in T = 15 and 60 min nutrient fluctuations induced growth rate responses, distinct from cells responding to single shifts, suggests the existence of a distinct physiology adopted by cells upon experiencing rapid nutrient fluctuations. Growth rate stabilized at comparable values in both fluctuating timescales (Fig. 4d), suggesting that T = 15 and 60 min nutrient fluctuations induce the same or very similar physiologies. Future work to access the proteomic and transcriptomic responses to fluctuations will be important to understand the molecular mechanisms of adaptation to fluctuations. Currently, our observations do not support the hypothesis that the reduction in growth rate under fluctuations results from continued physiological transitions toward growth at G high and G low. Instead, they led us to hypothesize that rapid fluctuations may induce a fluctuation-adapted physiology, one that remains stable across minute-scale changes in nutrient concentration.

Rapid nutrient fluctuations induce a fluctuation-adapted growth physiology. To demonstrate that bacteria can adopt a distinct physiological state when exposed to rapid nutrient fluctuations, we performed an experiment in which cells growing under steady C low for at least 3.5 h were then exposed to fluctuations of T = 60 min. We found that the first nutrient upshift induced the gradual increase in growth rate characteristic of the physiological transition between G low and G high observed from single-shift conditions (Fig. 5a). Subsequent upshifts displayed faster growth rate adjustments that increasingly resembled that characteristic of cells grown in fluctuating conditions (Fig. 5a), confirming that cells can adopt a fluctuation-induced growth physiology, induced by repeated nutrient shifts.

The transition to a stable growth physiology occurred within 2–3 h of the onset of fluctuations. Growth from each successive nutrient upshift (growth in C high) increased during this 2–3 h transition time, offsetting the decreasing growth following each successive downshift (growth in C low) (Fig. 5b), and ceased to differ significantly from the third period of nutrient fluctuation onwards (Fig. 5b). The overall increase in growth between the initial and stabilized periods suggests that adopting the fluctuation-induced physiology enhances growth in fluctuating environments. This enhancement is potentially a physiological trade-off that limits the maximum growth rate in any given nutrient concentration to increase the cell’s potential for growth when higher nutrient concentrations become available (Fig. 5b). Indeed, in the minutes after a nutrient shift, growth rate measured from cells in fluctuating conditions was higher than that of cells exposed to a single shift (Fig. 4f and Supplementary Fig. 9b). Thus, the stable physiology adopted by cells exposed to rapid nutrient fluctuations can alleviate the potential reduction of growth by unsteady conditions.

Growth in rapid nutrient fluctuations is higher than predicted by single-shift growth models. To quantify the advantages of a fluctuation-induced physiology, we compared mean growth rates under fluctuations with mean growth rates predicted by a null model. We constructed the null model from the growth rate dynamics quantified from single shifts (Fig. 4a), simulating growth rate over time under nutrient fluctuations while assuming single-shift dynamics (Fig. 6a). Two different assumptions produced different dynamics under minute-scale fluctuations, yet predicted the same trends (Supplementary Fig. 10 and Construction of Null Model in the Supplementary information). These dynamics were then averaged to predict G null in the absence of a fluctuation-induced physiology across a range of fluctuation.
timescales ($T = 30$ s to $96$ h) (Fig. 6b and Supplementary Table 5). For very slow fluctuations, the null model predicted that $G_{fluc}$ approaches the value predicted by Jensen’s inequality, $G_J$ (Fig. 6b), as cells spend almost the entirety of each nutrient phase at either $G_{low}$ or $G_{high}$ (Fig. 6a). Across all nutrient timescales $T$, the null model predicted that $G_{fluc}$ diminishes with decreasing $T$ (Fig. 6b).

This trend in the null model is the opposite of that displayed by the measured values of $G_{fluc}$ for rapid fluctuations. Experimentally, we measured an increase in $G_{fluc}$ with decreasing fluctuation period: $G_{fluc}$ is $50\%$ below $G_{ave}$ for $T = 60$ min, but only $16.5\%$ below $G_{ave}$ for $T = 30$ s (Fig. 6b). Relative to $G_{ave}$, the measured $G_{fluc}$ represents only $70.8 \pm 10.0\%$ of the predicted growth lost for $T = 5$ min and $38.9 \pm 10.8\%$ for $T = 30$ s (mean and standard deviation, $n = 3$). This increase in measured $G_{fluc}$ over the null model predictions represents the growth advantage in fluctuating conditions afforded by the fluctuation-induced growth physiology. These results demonstrate that growth rate in rapid fluctuations is not only quantitatively distinct from the values of $G_{fluc}$ predicted from single-shift dynamics, but also shows a qualitatively different trend in $G_{fluc}$ across nutrient timescales. While the values of $G_{fluc}$ predicted by the null model decreased with decreasing nutrient timescale, measured $G_{fluc}$ increased with decreasing nutrient timescale, suggesting that the cellular physiology induced by rapid fluctuations is an adaptation suited to alleviate some of the growth rate losses imposed specifically by second- and minute-scale nutrient fluctuations.

A remaining question concerns how cells sense that the environment is rapidly fluctuating and initiate the transition to a fluctuation-induced growth state. It is unclear how the fluctuation-induced physiology is achieved, in part because we do not know how gene expression or function may differ between cells growing in fluctuations and cells growing at steady state; however, prior work in bulk chemostat systems delivering transient minutes-long pulses of glucose may offer important insights. For example, in glucose-limited conditions, some yeast species (including S. cerevisiae) respond to a pulse of glucose by activating alcoholic fermentation, while others prevent ethanol production by increasing acetaldehyde oxidation. Yeasts that ferment the pulsed glucose into ethanol instead of biomass generally exhibit no change in growth rate across the pulse, whereas yeasts avoiding pulse-activated fermentation exhibit increased biomass production upon the addition of glucose. The dependence of growth rate in dynamic environments on the allocation of metabolic flux has also been proposed to occur in E. coli, which uses the carbon storage molecule glycogen to maintain higher growth rates under pulsed nutrient conditions. Experiments to test whether similar processes may explain our findings could be performed by adapting such bulk systems to measure gene expression and metabolic activity under minute-scale nutrient fluctuations.

Alterations in gene expression can be beneficial when environments change on rapid timescales. For example, increased expression of photoprotection proteins has been demonstrated to increase the growth yield of plants exposed to minute-scale fluctuations in light. Alternatively, post-translational activation of so-called spare ribosomes has been implicated as a bacterial strategy to increase growth rate rapidly (i.e., without new protein synthesis) upon nutrient upshift. E. coli, upon sensing depleted intracellular amino acid levels, has also been observed to induce broad responses to increase energy production, ribosome levels, and translational capacity. These observations illustrate the diversity of strategies that life has evolved to cope with the challenges of environmental change. By reporting a fluctuation-adapted physiology with growth benefits
in rapidly fluctuating environments, we contribute a framework by which to pursue an understanding of bacterial growth that is relevant to realistic habitats.

Accounting for fluctuations in models of bacterial growth. Our study is the first to observe single-cell responses to rapid nutrient fluctuations and report a fluctuation-induced growth physiology. We found that bacteria exposed to nutrient fluctuations exhibit reduced growth rates compared to that of the steady average nutrient condition, even when nutrients fluctuated on timescales as rapid as seconds. These reductions are not explained by the current paradigm in bacterial physiology, which holds that cells experiencing a shift in their nutrient environment will begin the transition to the steady-state growth physiology of the post-shift environment\(^{1,2,23-25}\). Despite the evidence that bacteria encounter rapid and repeated nutrient fluctuations in their environments\(^{1,2,4,5,8,37-41}\), single shifts in nutrient composition remain the dominant method by which bacterial growth is studied in dynamic nutrient conditions\(^{1,2,4,25}\). This study reports key differences in growth rate dynamics between fluctuations and single shifts, thus introducing repeated fluctuation as a better experimental system to study variability in microbial habitats than the classic single-shift paradigm.

In finding starkly different responses between fluctuations and single shifts, our study presents a conceptual advance important for the interpretation of past work and the direction of future work considering microbial growth in complex environments. By quantifying single-cell growth rate across a range of fluctuation timescales relevant to many bacterial habitats, this work demonstrates that growth responses to fluctuations are not simply transitions between two discrete states. Instead, we uncovered a strong dependence of bacterial growth on the temporal dynamics of nutrient concentration, highlighting the importance of temporal variability (and by extension, microscale heterogeneity) when considering bacterial growth in realistic environments. This work establishes nutrient timescale as a fundamental parameter characterizing bacterial environments, forming a third axis (in addition to chemical composition and temperature) to consider when studying bacterial growth.

The discovery of a fluctuation-induced growth physiology in \textit{E. coli} highlights the importance of temporal context in bacterial regulation of growth with nutrient availability. Further studies with diverse patterns of nutrient fluctuations may yield additional strategies of bacterial growth in temporally variable environments. It is also possible that distinct strategies of growth in complex temporal environments have evolved between bacterial species that occupy distinct ecological niches. Taxon-specific specializations for the timescale of light fluctuations have long been observed in plants\(^{42}\), and more recently in microbial response times to inputs of water to dry soils\(^{43}\).

Our results illustrate how identical environmental shifts can induce different responses depending on the timescale at which the shifts are delivered, demonstrating the need to account for temporal variability in the environment at timescales that have been mostly ignored to date. Understanding the diversity of growth responses to realistic features of microbial environments will bring us closer to the establishment of general frameworks for bacterial growth in natural ecosystems and the discovery of mechanistic links between the interactions that occur on the scale of single cells, populations, and communities.

Methods

\textbf{Bacterial strain.} All experiments in this study were performed with the same \textit{E. coli} strain, K-12 NC3722 ΔmotA. The motility mutant (ΔmotA) lacks flagella, facilitating long-term observation in microbiolites\(^{37}\), and is derived from the background strain K-12 NC3722, which lacks the growth defects observed in other strains of \textit{E. coli}\(^{44,45}\).

\textbf{Growth media.} \textit{Batch culture medium.} MOOPS (3-N-morpholino)propanesulfonic acid) medium (Teknova) supplemented with 0.2% glucose w/v and 1.32 mM K\textsubscript{2}HPO\textsubscript{4} was used for overnight and seed batch cultures. All batch cultures were 3 mL of supplemented MOOPS medium inoculated with \textit{E. coli}.

\textit{Microfluidics medium.} Lysogeny broth (LB) composed of tryptone (10 g/L), yeast extract (5 g/L), and NaCl (10 g/L) was used for all microfluidics conditions. For the microfluidics experiments, the same stock solution of 100% LB was mixed with an equimolar NaCl solution (2.5 g NaCl in 250 mL 0.22 µm filtered water; Millipore Millipak Express 40, catalog no. MGP04001) to prepare three dilutions: low, average, and high. To avoid bubble formation within the microfluidics, the NaCl solution was freshly autoclaved the day of each experiment and then cooled before preparing the LB dilutions. The high LB (2%) mixed 2 mL of the full LB into 98 mL salt solution. The low LB (0.1%) mixed 5 mL of the high LB solution with 95 mL salt solution. Afterward, the high LB solution was labeled with 0.26 nM sodium fluorescein, to allow visual calibration of switching between media. All solutions four of each nutrient concentration (\textit{C\textsubscript{low}}, \textit{C\textsubscript{ave}}, and \textit{C\textsubscript{high}}). Three of each concentration were inoculated with cells, while the fourth was kept bacteria-free and sampled as a blank control. The inoculum was prepared with the same overnight and seed culture preparation used for the microfluidics experiments (see \textit{Cell preparation}). Once the seed culture reached an \textit{OD}\textsubscript{600} of 0.1 (grown in MOOPS medium with 2% glucose), they were centrifuged for 2 min and 1 mL aliquots were centrifuged for 2 min. The media were loaded into plastic 10 mL syringes (Codan) or glass vials (VWR, cat. no. 548-0154) and warmed to 37 °C at least 3 h prior to the start of each experiment.

\textbf{Metabolomics characterization of growth media.} Changes in extracellular nutrient concentrations can affect uptake rates (due to variations in transporter affinity) or uptake order (in rich media, \textit{E. coli} have been observed to deplete preferred metabolites before beginning to consume others\(^{46}\)). To determine whether the preferred nutrient sources in our microfluidics experiments differed with nutrient concentration, we measured the depletion of extracellular metabolites from batch cultures. Batch cultures were necessary to observe metabolite depletion by enabling higher cell densities per volume. Measurements of depletion from the earliest time points after inoculating the batch cultures (i.e., when the media composition was least changed) best represented the nutrient consumption in microfluidic conditions, which continuously replenished all metabolites.

We collected the supernatant from 20 mL batch cultures grown at 37 °C with shaking in 125 mL Erlenmeyer flasks. Twelve flasks were prepared in parallel, four of each nutrient concentration (\textit{C\textsubscript{low}}, \textit{C\textsubscript{ave}}, and \textit{C\textsubscript{high}}). Three of each concentration were inoculated with cells, while the fourth was kept bacteria-free and sampled as a blank control. The inoculum was prepared with the same overnight and seed culture preparation used for the microfluidics experiments (see \textit{Cell preparation}). Once the seed culture reached an \textit{OD}\textsubscript{600} of 0.1 (grown in MOOPS medium with 2% glucose), they were centrifuged for 2 min and 1 mL aliquots were centrifuged for 2 min. The media were loaded into plastic 10 mL syringes (Codan) or glass vials (VWR, cat. no. 548-0154) and warmed to 37 °C at least 3 h prior to the start of each experiment.

\textbf{Methods}
next morning, cells from the overnight culture were diluted to achieve 3 mL of supplemented MOPS medium with an initial OD<sub>600</sub> below detection, generally a 1:1000 or 1:2000 dilution. This seed culture was used to inoculate microchannels once cells reached an OD<sub>600</sub> between 0.07 and 0.10.

**Microchannel fabrication.** Microfluidic channels with a depth of 60 µm were cast in polydimethylsiloxane (PDMS) from a custom-made master mold such that all four channels (one microfluidic signal generator (MSG) for fluctuating environments and three straight channels for steady environments) were present on the same device (Fig. 1a). Each PDMS device was bonded to a glass slide by plasma treating each interacting surface for at least 1 min, then incubating the assembled chip for at least 2 h at 80 °C. The morning of each experiment, bonded channels were cooled to room temperature and then treated with a 1:10 dilution of poly-l-lysine (Sigma, catalog no. P8920) in milliQ water. Poly-l-lysine treatment increased the number of attached cells. This attachment duration, allowing for longer adherence of single cells without affecting growth (Supplementary Fig. 2b, c). This treatment has no effect on the growth rate (Supplementary Fig. 2c) and involves incubating the diluted poly-l-lysine solution inside each channel for 15 min, before gently removing the solution and flushing the emptied channel with sterile milliQ water. The treated channels were then air-dried for at least 2 h prior to experimental use.

**Nutrient signal generation and calibration.** All fluctuating nutrient signals in this study switched between two nutrient media: a high and low concentration of LB. To switch between media, we oscillated pressure within each reservoir of the nutrient medium while maintaining a steady mean pressure to ensure a steady total flow rate (Supplementary Fig. 1). This flow rate was determined by collecting the fluid output from the MS and measuring the volume per minute. While the pressure differentials across the setup can vary (e.g., different device, slight variations in tubing lengths and angles), the range of flow rates used had no effect on growth rate (Supplementary Fig. 2). Because the pressure differentials could vary from day to day, the pressure differences required to completely switch between media were calibrated prior to each experiment at ×20 magnification, which enabled visualization of the entire signal junction (Supplementary Fig. 1). These calibrated pressure differences were then used to define the fluctuating nutrient signal. The pressure system was programmed to generate the signal in synchrony with image acquisition. Thus, timestamps from the image data could be directly correlated with specific time points within the nutrient signal. Separately, the stability of the calibrated signal was visually confirmed by comparing the fluorescent signal exiting the junction with the signal observed downstream (Fig. 1c and Supplementary Fig. 3). These visualizations were conducted at ×60 magnification and quantified by custom image processing scripts in MATLAB (see Code availability).

**Quantification of switching timescale and nutrient signal stability.** To assess the correspondence between our designed signal (an even square-wave) and the signal realized within the microfluidic device (Fig. 1c), we compared the transition dynamics of nutrient shifts occurring immediately after the signal junction and those occurring near the end of the cell imaging region. Two positions in the MSG were imaged while the fluid flowing through fluctuated between a medium labeled with 0.26 mM sodium fluorescein and an unlabeled medium on a 30 s period. Specifically, we compared (1) the sharpness of the signal immediately upstream (i.e., with the observed modulation with that observed further downstream, as experienced by the surface-attached cells), and observed virtually no decay in the fluorescent signal between the signal junction and the imaging region (Supplementary Fig. 3), only the time delay as calculated in Supplementary Table 1. While the fluid flow occurs in slow motion—was it able to capture the diffusion of nutrients (or sodium fluorescein, which is 2–4 times the molecular weight of an amino acid) could potentially smooth out our nutrient signal. Diffusion can be further aided by Taylor dispersion, a phenomenon in which velocity gradients in the fluid flow (i.e., shear) work to increase the effective diffusion of a chemical species by spreading it across a larger region, thereby favoring diffusion. Were the magnitude of these effects non-negligible in our system, we should expect different slopes during transitions between the signals at the junction and downstream. Specifically, the downstream signal should have a longer transition time as we would detect fluorescence leaking into the C<sub>low</sub> phases of the signal. However, the time required to completely transition (i.e., time to go from baseline to saturated fluorescent signal and vice versa) was about 2 s in both locations (Supplementary Fig. 3). Thus, our flow rates are sufficiently fast to carry our intended signal across the entire length of the device without noticeable smoothing from diffusion. We also determined that the periodic oscillations in the nutrient signal are robust across time. The programmed period (T = 30 s) was reliably quantified between peaks and between troughs from the repetitive fluorescent signal (Supplementary Fig. 3).

**Microfluidics experimental procedure.** The system used to operate the MSG involves: (1) a Nikon Eclipse Ti inverted microscope, (2) a full-case incubator that maintains a stable temperature (37 °C) around the entire microscope, except for the camera and light sources, (3) a computer to operate the microscope software (Nikon Elements) and MATLAB, (4) a data acquisition (DAQ) device that interfaces with MATLAB to control two pressure regulators, one for each nutrient source, (5) two reservoirs of nutrient medium, one of each nutrient concentration, and (6) a source of compressed air. The compressed air is fed into the pressure system through a manually adjustable regulator, which caps the pressure directed toward the two automated regulators at 1.5 psi. To ensure that the automated regulators receive a stable input, the pressure of the compressed air source is higher than this maximum value. Each automated regulator is connected to and modulates the internal pressure of one reservoir of nutrient medium (C<sub>high</sub> or C<sub>low</sub>). Each nutrient reservoir is a septum-capped glass vial (vWR, cat. no. 547-9154; cap: VWR, cat. no. 547-5872) with two needles inserted into the silicone septum: one short and one long. The short needle directly connects an automated pressure regulator with the air space within its reservoir, thereby adjusting the pressure within the reservoir as dictated by the MATLAB signal (Supplementary Fig. 1a). The long needle connects the fluid within its reservoir with the microchannel via tubing inserted into the inlet of the device (Supplementary Fig. 1a). The microscope and media are contained within a custom LIS incubator, which maintains the sample and all media at 37 °C.

Experiments were based on the exposure of cells attached to the lower surface of the microchannels to precisely controlled fluctuating or steady nutrient conditions, and the imaging of thousands of cells in the downstream imaging region in order to calculate their individual growth rates. The treated, dry microchannels were inoculated with ~50 µL of the seed culture (see Cell Preparation) for 10–15 min, allowing cells to settle and attach to the glass surface within each microchannel (Supplementary Fig. 1a). The microfluidic device was placed in a vacuum for at least 10 min to remove air from the PDMS. This step helped to avoid the presence of bubbles inside the channels, by removing air from the PDMS so that any air introduced in the setup would be absorbed by the PDMS. Inputs to fluctuating conditions were two septum-capped glass vials (each for high and low nutrient) from which flow was driven by a custom-built air pressure system (Fig. 1). Signals to steady conditions were driven by plastic syringes (Codan) from which flow was driven by a syringe pump (Harvard Apparatus). Outputs for all conditions led to liquid waste receptacles. To avoid changes in pressure throughout experiments, we ensured that the waste tubing was sufficiently short to never become submerged by the rising level of media waste.

**Image acquisition.** Individual cells from all microchannel environments were imaged with phase-contrast microscopy using a Nikon Eclipse Ti inverted microscope equipped with an Andor Zyla sCMOS camera (6.5 µm per pixel) at ×60 magnification (×40 objective with an additional 1.5×s), for a final image resolution of 2.6 µm per pixel. This magnification was high enough to image changes in growth between each image, yet low enough to image hundreds of cells per field of view. Each position was repeatedly imaged every 117 s (1:57 min), a time step sufficiently short to allow the acquisition of multiple time points along a growth curve (i.e., 10 time points in a 20 min cell cycle), yet infrequent enough to image a total of 40–50 positions within each time step. Imaging 10–15 positions with each of four parallel conditions (i.e., fluctuating and steady C<sub>high</sub>, C<sub>low</sub>, C<sub>low</sub>, C<sub>low</sub>) required roughly 1.5 min. Generally, ten imaging positions per condition allowed us to track 300–1000 or more cells per nutrient condition. We confirmed that growth rates were independent of a cell’s position along the 10-mm-long region of the microchannel within the ±15 µm region of the camera frame. Cells experienced identical nutrient time series, regardless of location within the microchannel. The uneven time step (1:57 min as opposed to 2:00 min) was chosen to avoid potential aliasing effects, by sampling at various points along the nutrient period instead of repeatedly at the same few light. Light exposure was limited to 20 ms per image, with the shutter opened during image capture. Image acquisition was fully automated through Nikon Elements, supplemented with the Nikon Perfect Focus System to prevent loss of focus due to vertical shifts in the sample.

**Image processing.** In preparation for analysis, image sequences from microfluidic experiments were first passed through a particle tracking step and a quality control step. First, a custom MATLAB particle tracking pipeline was developed to (1) read image data directly from Nikon Elements, (2) read image data from files (for postprocessing based on pixel intensity), (3) fit an ellipse to each particle and measure particle parameters (e.g., length, width) and (4) track individual particles through time. Second, to generate errors from our analysis—for example, particles arising from noise (e.g., non-uniformity in the background) or particles that include more than one cell—a quality control step trimmed our tracked dataset, using standard deviation filters to exclude errors. The parameter values used in both steps ensured that 93–97% (depending on nutrient condition) of tracks derive from isolated single cells. The final output of these two steps is a data matrix containing parameter data (e.g., cell length) over time for hundreds of individual cells growing in isolation. Reducing our analysis to cells without neighbors allowed us to assess conditions on cell proliferation or death of medium components, and no physical interactions between cells. Cells in contact were excluded from the analysis to avoid the possibility of metabolic interactions and imprecision in the measurement of cell size. Specifically regarding the particle tracking and quality control steps are available in the scripts (see Code availability). Images in figure panels were adjusted for visualization using Fiji.49
Quantification and statistical analyses
Calculating instantaneous growth rate, \( \mu \). One widely used method to calculate growth rate is to consider single-cell growth an exponential process\textsuperscript{12,18} and solve for instantaneous growth rate, \( \mu \). This definition of growth rate is used throughout this study. From the length and width measured during particle tracking, the instantaneous volume of each individual cell was approximated as a cylinder with hemispherical caps\textsuperscript{19}. The approximated volumes were then used to compute instantaneous single-cell growth rates in terms of volume doublings per hour. Using \( V(t + \Delta t) = V(t) \times 2^{\Delta t} \), we calculated \( \mu \) between each pair of time steps, with \( \Delta t = 117 \text{ s} \) (image frame rate). Specifically, we took the natural logarithm of each volume trajectory and calculated the slope between each point. Dividing the slope by the natural log of 2 changes the base of the exponential from \( e \) to 2. Thus, \( \mu \) represents the exponential rate at which volume doubles.

Accounting for day-to-day variability in growth rate. While steady-state growth rates were generally reproducible (Supplementary Fig. 5b and Supplementary Table 6), we found that growth rate measurements performed on the same day (i.e., same seed culture) were moderately correlated (Supplementary Table 7). This correlation indicates that slight differences between the seed culture (which was different for each experiment) contributed to the differences in growth rate measured from identical conditions between experiments. Thus, when comparing growth rate across conditions (e.g., \( \text{G}_{\text{ave}} \) and \( \text{G}_{\text{max}} \)), we compared measurements performed on the same day before comparing between experimental replicates, calculating the fraction of \( \text{G}_{\text{ave}} \) represented by the measured \( \text{G}_{\text{ave}} \) from that same experiment before calculating statistics (i.e., mean and standard deviation of \( \text{G}_{\text{ave}}/\text{G}_{\text{max}} \)) across experiments. We used this same approach when comparing \( \text{G}_{\text{max}} \) to \( \text{G}_{\text{ave}} \), which was calculated from each experiment’s \( \text{G}_{\text{ave}} \) and \( \text{G}_{\text{max}} \) and \( \text{G}_{\text{ave}} \) to \( \text{G}_{\text{max}} \). The alternative approach for this comparison would be to calculate the mean and standard deviation between experimental replicates before calculating fractions (e.g., \( \text{G}_{\text{ave}}/\text{G}_{\text{max}} \)) and combining the error. Numerically, this alternative approach yields very similar results.

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Raw mass spectral data is deposited in massIVE and accessible with the accession code MSV000087096. The KEGG database used to annotate mass spectral data is available at https://github.com/jkimthu/under-pressure. Raw mass spectral data is deposited in massIVE and accessible with the accession code MSV000087096. The KEGG database used to annotate mass spectral data is available at https://github.com/jkimthu/under-pressure. Raw mass spectral data is deposited in massIVE and accessible with the accession code MSV000087096. The KEGG database used to annotate mass spectral data is available at https://github.com/jkimthu/under-pressure. Raw mass spectral data is deposited in massIVE and accessible with the accession code MSV000087096. The KEGG database used to annotate mass spectral data is available at https://github.com/jkimthu/under-pressure.

Code availability
Image processing, data analysis, and plotting scripts are available at https://github.com/skimthu/growing-up\textsuperscript{31}. Scripts to automate fluctuating signal generation are available at https://github.com/skimthu/under-pressure\textsuperscript{32}.

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Author contributions

J.N., V.F., M.A., and R.S. designed the study; J.N. and V.F. designed the microfluidic system, pressure control system/software, and particle tracking software; J.N. performed the microfluidic experiments and analyzed the growth data; S.P. performed MS measurements and analyzed the metabolomics data; U.S. provided an overview on metabolomics data generation and analysis; J.N., M.A., and R.S. wrote the paper; all authors reviewed the paper before submission.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to R.S.

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