Ecological suicide in microbes

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The growth and survival of organisms often depend on interactions between them. In many cases, these interactions are positive and caused by a cooperative modification of the environment. Examples are the cooperative breakdown of complex nutrients in microbes or the construction of elaborate architectures in social insects, in which the individual profits from the collective actions of her peers. However, organisms can similarly display negative interactions by changing the environment in ways that are detrimental for them, for example by resource depletion or the production of toxic byproducts. Here we find an extreme type of negative interactions, in which Paenibacillus sp. bacteria modify the environmental pH to such a degree that it leads to a rapid extinction of the whole population, a phenomenon that we call ecological suicide. Modification of the pH is more pronounced at higher population densities, and thus ecological suicide is more likely to occur with increasing bacterial density. Correspondingly, promoting bacterial growth can drive populations extinct whereas inhibiting bacterial growth by the addition of harmful substances—such as antibiotics—can rescue them. Moreover, ecological suicide can cause oscillatory dynamics, even in single-species populations. We found ecological suicide in a wide variety of microbes, suggesting that it could have an important role in microbial ecology and evolution.

Microbes not only depend on their environment but also modify it. An important environmental parameter for microbial growth is pH, because protein and lipid membrane stability depend strongly on the pH of the environment. Microbes have a species-dependent pH optimum at which they grow best and an environmental pH above or below this optimum inhibits growth or can even cause cell death. At the same time, bacteria change the environmental pH by their metabolic activities. In this way, microbes can potentially induce pH values that are detrimental for their own growth and thus harm themselves.

Results

The soil bacteria species Paenibacillus sp. (most similar to Paenibacillus tundae, for more information about this strain see Supplementary Information) can grow in a medium that contains 1% glucose as the main carbon source, in addition to a small amount of complex nutrients (see Methods for details). In soil, the amount of carbohydrates ranges from 0.1% to 10%, mostly in the form of complex carbohydrates. Starting from neutral pH, we measured strong acidification of the environment to a pH of around 4 during bacterial growth due to the secretion of a variety of organic acids (Fig. 1a, Supplementary Fig. 1b). Upon reaching this low pH, the bacteria suddenly started to die, resulting in a non-monotonic growth curve (Fig. 1a), since Paenibacillus sp. cannot survive at low pH values (Supplementary Figs. 1a and 3). Indeed, after 24 h of incubation, we found that there were no viable cells in the culture (as measured by colony forming units (CFU) after 48 h incubation on rich medium, which may exclude cells that could grow after more than 48 h). We note that the bacterial densities that are reached in these experiments are within the range that can be found in soil and soil has a slightly lower buffering capacity than our medium (Supplementary Fig. 2). Moreover, ecological suicide also appears on non-glycolytic substrates (such as glycerol) and complex sugars such as starch (Supplementary Fig. 1). We call this rapid population extinction due to environmental modification ecological suicide—a phenomenon that has previously been hypothesized.

The correlation between the drop of pH and the onset of death suggests that the bacteria themselves may be responsible for their eventual extinction by lowering the pH into regions in which they cannot survive. To test this idea, we added buffer to the medium to temper the pH change. The buffer indeed slows down the death process (Fig. 1b) and prevents it completely at sufficiently high concentrations (Fig. 1c). Thus, it is the pH change that causes the death of the bacteria and the presence of buffer can hinder ecological suicide. These results show that initially flourishing bacterial populations can corrupt their environment and thus cause their own extinction. The pH change resembles a ‘public bad’ that is collectively produced and harms all members of the population. This phenomenon can be recapitulated by a simple mathematical description based on negative feedback between the bacteria and the environmental pH (Supplementary Discussion and Supplementary Fig. 10).

Because the bacteria collectively change the pH, higher bacterial densities can deteriorate the environment more strongly and thus expedite ecological suicide. We tested this idea experimentally by measuring the fold growth within 24 h for different initial bacterial densities and different buffer concentrations. At low buffer concentrations, the bacteria die by ecological suicide independent of their initial density, whereas at high buffer concentrations they always survive (Fig. 2a). At intermediate buffer concentrations, however, survival becomes density dependent (Fig. 2a). For high initial cell densities, the bacteria die within 24 h, but below a critical initial density, the bacteria grow and survive. The fitness of the bacteria thus decreases markedly with increasing cell density. This aspect of ecological suicide is thus opposite to the well-known Allee effect, in which fitness increases with population density. Although the observed death at high cell densities is reminiscent to death at high densities in common logistic growth models, in our experiments death continues until all cells have died out, whereas in logistic growth the density stabilizes at the carrying capacity.

What does this growth behaviour mean for the long-term growth dynamics of population such as those that occur during growth in medium with daily dilution into fresh medium? Figure 2a shows...
how the bacterial density after one day of growth depends on the initial bacterial density. With intermediate buffering, the bacteria die for high initial densities but grow for low initial densities. This may cause oscillatory dynamics, since high bacterial densities cause low densities on the next day and vice versa. Indeed, this intuitive prediction is fully supported by a mathematical description based on negative feedback of the bacteria and the environmental pH alone, which shows a bifurcation of the end-of-the-day bacterial densities upon changing the buffer concentration (see Supplementary Information and Supplementary Figs. 11 and 12). To test this prediction, we cultured the bacteria in batch culture with a daily 1:100 dilution of the culture into fresh medium. As expected from Fig. 2a, with low buffering the bacteria go extinct on the first day and with high buffering they grow up to the same saturated density each day (Fig. 2c,c, Supplementary Figs. 6 and 8a). With intermediate buffering, however, the bacteria show oscillatory dynamics as predicted by our mathematical model (Fig. 2d, Supplementary Figs. 8b and 10).

The oscillations in the population densities are accompanied by oscillations in the time at which the pH drops each day (acidification time, Fig. 2d and Supplementary Figs. 5 and 8b), which again shows the connection between pH change and ecological suicide. Ecological suicide caused by environmental deterioration therefore can drive oscillatory dynamics even in populations that consist of only one species.

We have seen that low bacterial densities lead to less deterioration of the environment and thus a less deadly effect on the bacteria. Therefore, effects that hinder bacterial growth by harming the bacteria may be able to save the population from ecological suicide. A first indication in this direction was found when changing the glucose concentration. Although one would expect that an increase in glucose concentrations is beneficial, in the presence of ecological suicide, the opposite is the case (Fig. 3a). At low glucose concentrations, the bacteria grow to lower densities, which hardly changes the pH and therefore allows the bacteria to survive. At high glucose concentrations, bacterial growth causes environmental acidification and thus ecological suicide. The bacterial population is therefore only able to survive in nutrient-poor conditions. Moreover, Figure 3a shows that ecological suicide can be observed even at rather low nutrient concentrations of around 0.2% glucose.

To explore the idea that environments that are usually considered poor can instead save the bacterial population, we measured the growth and survival of bacteria grown in the presence of the antibiotic kanamycin, or in the presence of ethanol or salt. Although these substances are different, they all inhibit bacterial growth and lead to similar profiles of population survival as a function of the concentration of the inhibiting substance (Fig. 3b–d). In the absence of harmful substances, the bacteria lower the pH to the point of extinction. At high concentrations, the harmful substances kill the bacteria. However, at intermediate concentrations, the bacteria can grow and survive. This leads to the paradoxical situation that substances that are normally used to kill bacteria in medicine (antibiotics) or food preservation (salt, ethanol) are able to save bacteria and enable their growth. The interplay between the harming substance and the ecological suicide results in a U-shaped dose–response curve of the harming substance, which is called hormesis in toxicology.

The effect of ecological suicide is surprising and has paradoxical consequences. However, the question arises: how common is ecological suicide in bacteria? To investigate this question, we incubated 119 bacterial soil isolates from a broader taxonomic range (Supplementary Fig. 9) in the presence of glucose as a carbon source and urea as a nitrogen source. Glucose can be converted to organic acids and this acidifies the medium, whereas urea can be converted by many bacteria into ammonia and this alkalizes the environment. From these 119 strains, the 21 strongest pH modifiers (either in acidic or alkaline directions) were tested for the presence of ecological suicide by measuring the fold growth in 24 h at low and high buffer concentrations (Fig. 4a). Indeed, around 25% of the strains suffered ecological suicide and were unable to survive at low buffer concentrations yet could be saved by more buffering (Fig. 4b).

Another 20% grew better at high than low buffering, suggesting a
Ecological suicide can cause oscillations in the population size over time. **a** At a low buffer concentration (10 mM phosphate), the bacteria commit ecological suicide, whereas at a high buffer concentration (100 mM phosphate), the bacteria grow, in both cases independent of their initial density. However, at a moderate buffer concentration (26 mM phosphate), the bacteria die at high starting densities and grow at low starting densities. The fold growth at a high buffer concentration decreases for increasing initial bacterial densities, since the final bacterial density equals the carrying capacity and is therefore constant. Mean (solid lines) and s.e.m. (error bars) are shown for four replicates. The black horizontal dashed line corresponds to a fold growth of 1. **b** To explore long time growth dynamics, the bacteria were grown in a daily dilution scheme with 24 h of incubation in well-mixed conditions followed by a 1:100 dilution into fresh medium. **c-e** At low (10 mM phosphate; **c**) and high (100 mM phosphate; **e**) buffer conditions, the bacteria either die on the first day or grow to saturation every day. **d** However, at medium buffer conditions, we measure oscillatory dynamics of the bacterial density. This is accompanied by oscillations in the time that the bacteria need to acidify the environment (acidification time, Supplementary Fig. 8). The exact type of oscillatory dynamics depends on the slope and shape of the curve in **a**, as discussed in more detail in the Supplementary Information. **c-e**, The four blue lines (solid, dashed, dotted, dashed-dotted; the separated curves can be seen in Supplementary Fig. 5) show different replicates. The strong differences between the replicates highlight the sensitivity of these oscillations to experimental conditions and that they probably do not show a limit cycle oscillation.

**Discussion**

We demonstrated that microbes are able to cause their own extinction by deteriorating the environment, a process that we call ecological suicide. Several cases are described in which microbial populations experience a slow decline after reaching saturation. However, this decline is usually very slow compared to the growth rate and does not cause sudden population extinction. In ecological suicide, however, the population does not even reach saturation; instead, the bacteria switch immediately from a growth into a death phase (Fig. 1a). A notable exception are quorum sensing deficient mutants of several *Burkholderia* species that show a type of ecological suicide, whereas in the wild-type strains quorum sensing mediates a change in metabolism that avoids ecological suicide. This shows that bacteria can possess mechanisms that actively counteract ecological suicide.

A phenomenon similar but not identical to ecological suicide is population overshoot, which is often connected to overexploitation of natural resources and has been proposed in several macro-organisms, but it is mostly discussed in humans that overexploit the environment. Several ancient civilizations are suspected to have collapsed by overexploitation of natural resources. Upon overshoot, a population exceeds the long-term carrying capacity of its ecosystem, followed by a drop of the population below the carrying capacity, which usually does not lead to extinction of the population but is followed by recovery at a lower density. However, in our case of ecological suicide, the carrying capacity of the ecosystem is changed to zero—the bacteria produce a deadly environment and go extinct without recovery, which marks ecological suicide as an extreme version of population overshoot.

With daily dilutions, ecological suicide can result in oscillatory behaviour. Oscillations in ecology have been intensely studied, often as a consequence of species interactions in our system the second species is replaced by the pH value, resulting in a situation in which interactions between one species and its environment drive the oscillations. In a similar way, modification of and reacting to the environment have recently been described to cause metabolic oscillations in yeast, expanding waves in microbial biofilms or oscillations in populations densities by toxin production or resource competition.

In view of the high frequency of ecological suicide that we observed in natural isolates of soil bacteria, this effect may have a broad impact on microbial ecosystems in terms of microbial interactions and biodiversity and its occurrence and ecological meaning in nature have to be investigated in the future. Moreover, ecological suicide can happen on different carbon sources, at a lower temperature of 22 °C—although sufficiently low temperatures may stop ecological suicide—and even with complex sugars and thus under conditions that more resemble those in the soil (Supplementary Fig. 1). In our case, the ecological suicide was mediated by the pH, but changing any environmental parameter, such as oxygen levels...
or metabolite concentrations in self-harming ways may cause similar outcomes.

Our findings raise the question of how such self-inflicted death of microbes can exist without evolution selecting against them. We hypothesize that, although ecological suicide is detrimental for the population, it may be evolutionary beneficial for the individual bacterium. A fast metabolism of glucose may harm and even kill the population. The phenomena of ecological suicide could therefore be an end-product of evolutionary suicide. Future work should explore the evolutionary origin of ecological suicide as well as the consequences of this phenomenon for the ecology and evolution of microbes.

Methods
All chemicals were purchased from Sigma-Aldrich (St Louis, USA), if not stated otherwise.

Buffer. For precultures of the bacteria, the basic buffer recipe was 10 g/l yeast extract and 10 g/l soytone (both Becton Dickinson, Franklin Lakes, USA). We refer to this buffer as 1x nutrient medium (also 1xNu). The initial pH of this medium was 7 and 100 mM phosphate was added. For the washing steps and the experiments itself the medium contained 1 g/l yeast extract and 1 g/l soytone, 0.1 mM CaCl₂, 2 mM MgCl₂, 4 mg/l NiSO₄, 50 mg/l MnCl₂ and 1x Trace Element Mix (Teknova, Hollister, USA). We refer to this buffer as the base buffer. It was supplemented with phosphate buffer and/or glucose as outlined in the different experiments. The usual concentration was 10 g/l glucose, deviations from this concentration are described for the different experiments below. All media were filter-sterilized.

Estimation of CFU. To estimate the number of living bacteria in the different experiments we used colony counting. At the end of every growth cycle, a dilution row of the bacteria was made by diluting them once 1:100 and six times 1:10 in phosphate-buffered saline (PBS; Corning, New York, USA). With a 96-well pipette 10 µl of the bacteria was made by diluting them once 1:100 and six times 1:10 in phosphate-buffered saline (PBS; Corning, New York, USA). With a 96-well pipette 10 g/l (Viaflo 96, Integra Biosciences, Hudson, USA) 100 µl of every well for every dilution step was transferred to an agar plate (Tryptic Soy Broth (Teknova, Hollister, USA), 2.5% agar (Becton Dickinson, Franklin Lakes, USA) with a 150-mm diameter. The experiments were carried out in 5 ml medium in 50 ml culture tubes (Falcon, Becton Dickinson, Franklin Lakes, USA) overnight in 1x Nu with an additional 100 mM phosphate. The shaking speed was 250 r.p.m. on a New Brunswick Innova 2100 shaker (Eppendorf, Haupauge, USA), the lids of the Falcon tubes were only slightly screwedin to enable gas exchange. Except for the 24h experiment with hourly measurements, which were done in 50 ml culture tubes (Falcon, Becton Dickinson, Franklin Lakes, USA), the experiments were all done in 500-µl 96-deepwell plates (Deepwell Plate 96/500µl, Eppendorf, Haupauge, USA) covered with two sterile AeraSeal adhesive sealing films (Excell Scientific, Victorville, USA), the plates were shaken at 1,350 r.p.m. on Heidelberg platform shakers (Titramax 100, Heidelberg North America, Elk Grove Village, USA). The culture volume was 200 µl if not droplets were allowed to dry and the plates were incubated at 30 °C for 1–2 days until clear colonies were visible. The different dilution steps ensured that a dilution could be found that enabled the counting of colonies.

pH measurements. To measure the pH directly in the bacterial growth culture at the end of each growth cycle, a pH microelectrode (N60089NC, SI Analytics, Weilheim, Germany) was used. The grown bacterial cultures were transferred into 96-well PCR plates (VWR, Radnor, USA) that enabled the measurement of pH values in less than 200 µl.

Bacterial culture. All cultures were incubated at 30 °C. The precultures were carried out in 5 ml medium in 50 ml culture tubes (Falcon, Becton Dickinson, Franklin Lakes, USA) overnight in 1x Nu with an additional 100 mM phosphate. The shaking speed was 250 r.p.m. on a New Brunswick Innova 2100 shaker (Eppendorf, Haupauge, USA), the lids of the Falcon tubes were only slightly screwedin to enable gas exchange. Except for the 24h experiment with hourly measurements, which were done in 50 ml culture tubes (Falcon, Becton Dickinson, Franklin Lakes, USA), the experiments were all done in 500-µl 96-deepwell plates (Deepwell Plate 96/500µl, Eppendorf, Haupauge, USA) covered with two sterile AeraSeal adhesive sealing films (Excell Scientific, Victorville, USA), the plates were shaken at 1,350 r.p.m. on Heidelberg platform shakers (Titramax 100, Heidelberg North America, Elk Grove Village, USA). The culture volume was 200 µl if not...
Density dependence of growth. Density-dependence experiments are shown in Fig. 2a. The 96-deepwell plates were prepared by adding 200 µl base buffer containing 10 g l$^{-1}$ glucose and different phosphate concentrations ranging from 10 to 100 mM (see Table 1). To obtain different growth density the bacteria were added by different dilutions ranging from 1:10 to (1/4)$^5$:10 dilution. The 96-deepwell plates were incubated at 30 °C, 1,350 r.p.m. shaking. At the beginning of the experiment as well as after 24 h, the CFU was estimated. After 24 h, the pH was measured. For every condition there were two biological replicates and two technical replicates.

Growth under daily dilution. Daily dilution experiments are shown in Fig. 2b–d. The 96-deepwell plates were prepared as for the ‘density dependence of growth’ experiment. The bacteria were added by 1:100 dilution. The 96-deepwell plates were incubated at 30°C, 1,350 r.p.m. shaking. At the beginning of the experiment as well as after 24 h, the CFU was estimated. After 24 h, the pH was measured. Every 24 h, the CFU was estimated, the pH was measured and the bacteria and the fluorescence of the nanobeads was measured as for the 500-µl 96-deepwell plate, this 96-well plate was diluted 1:100 into fresh medium. To study the dynamics of bacterial growth and the pH, at the beginning of each day, the bacteria were also diluted 1:100 into a 96-well plate (96 Well Clear Flat Bottom Tissue Culture-Treated Culture Microplates, 353072, Falcon, Corning, USA) with the same medium in each well as for the 500-µl 96-deepwell plate. In addition, every well was supplemented by fluorescent nanobeads (1:100 dilution), which we fabricated as described below. In parallel to the incubation of the 500-µl 96-deepwell plate, this 96-well plate was then analysed in a Tecan infinite 200 Pro (Tecan, Männedorf, Switzerland) at 30 °C, 182 r.p.m., 4 mm amplitude. For this experiment, the optical density was measured by absorbance and the fluorescence of the nanobeads was measured by exciting fluorescein (excitation wavelength 450 nm, emission wavelength 516 nm) and tetrakis(pentafluorophenyl) porphyrin (TFPP; excitation wavelength 582 nm, emission wavelength 658 nm). Measuring the optical density of the bacteria and the fluorescence of the nanobeads every 15 min over the course of one day enabled us to study the change in pH. Although the optical density and fluorescence were measured in parallel growing 96-well plates, we argue that they (at least qualitatively) capture the dynamics in the 96-deepwell plate, which is underlined by the fact that the measured acidification time and bacterial density oscillate synchronous (Fig. 2d). Parallel to the oscillations in the CFU observed in the 96-deepwell plates, the fluorescence measurements in the 96-well plates display oscillations in the timepoint, the pH drops, that is, the timepoints of the fluorescence intensity's turning points (see Fig. 2d, Supplementary Fig. 2). For every buffer condition there were four biological replicates in the 96-well and 96-deepwell plates.

Fabrication of fluorescent nanobeads. To study the change in pH during our daily dilution experiments, we fabricated fluorescent nanobeads following a previously established protocol16. These nanobeads contain fluorescein, for which the fluorescence intensity depends on the pH, and a highly photostable fluorinated porphyrin (TFPP), which acts as a red-emitting reference dye. Because the fluorescence intensity of TFPP is independent of pH, it serves as internal standard to make the result independent of the overall nanobead concentration. Thus the ratio of the fluorescence of TFPP is independent of the pH value (Supplementary Fig. 4).

Effect of harmful conditions on bacterial survival. Survival experiments are shown in Fig. 3. The preculture was grown overnight in 1× Nu, pH 7 with 100 mM phosphate. After 15 h, the bacteria were diluted 1:100 into the same medium. Upon reaching an OD cm$^{-1}$ of 2, the bacteria were washed twice with base buffer and the OD cm$^{-1}$ was adjusted to 2. The bacteria were diluted 1:100 into 96-deepwell plates (Eppendorf, Hauppauge, USA) containing base medium, pH 7 with 10 g l$^{-1}$ glucose and different amounts of kanamycin, NaCl or ethanol. For Fig. 3a, the glucose concentration was varied. The bacteria were incubated for 24 h at 30°C, 1,350 r.p.m. on a Heidolph platform shakers (Titramax 100, Heidolph North America, Elkovle Village, USA) as described above. The live cell density was estimated by colony counting at the start of the experiment and after 24 h. The pH was measured after 24 h with a pH microelectrode as described above.

Frequency of ecological suicide. For the experiment shown in Fig. 4, 21 different soil bacteria were used, which were identified out of 119 soil bacteria to yield the highest change in pH. The 119 bacterial strains were isolated from a single grain of soil collected in September 2015 in Cambridge, Massachusetts, USA. The grain weighed approximately 1 mg and was handled using sterile technique. The grain was washed in PBS and serial dilutions of the supernatant were plated on nutrient agar (0.3% yeast extract, 0.5% peptone, 1.5% bacto agar) and incubated for 48 h at room temperature. Isolated colonies were sampled and cultured at room temperature in 5 ml nutrient broth (0.3% yeast extract, 0.5% peptone) for 48 h. To ensure purity, the liquid cultures of the isolates were diluted in PBS and plated on nutrient agar. Single colonies picked from these plates were once again grown in nutrient broth for 48 h at room temperature and the resulting stocks were stored in 20% glycerol at −80°C. The 16S rRNA gene was sequenced using Sanger sequencing of DNA extracted from glycerol stocks carried out at GENEWIZ (South Plainfield, New Jersey, USA). Sequencing was performed in both directions using the company's universal 16S rRNA primers, yielding assembled sequences around 1,100 nt in usable length. Some of those strains have been in more detail investigated previously.

In order to identify the 21 species that caused the strongest pH change, the precultures of the 119 soil bacteria were done in 200 µl 1× Nu, pH 7 with 100 mM phosphate for 14 h at room temperature, 800 r.p.m. shaking. The precultures were then diluted 1:100 into the same medium and grown for 6 h, which approximately corresponded to a growth to an OD cm$^{-1}$ of 2 for the precultures of Paenibacillus sp. used in the Paenibacillus sp. experiments described above. The bacteria were then diluted 1:100 into fresh medium and grown for 24 h at room temperature, 800 r.p.m. shaking. After 24 h, the bacterial density (CFU µl$^{-1}$) and pH of all cultures were measured and the 21 bacteria with the highest change in pH were selected.

For these 21 species, precultures were done in 5 ml 1× Nu, pH 7 with 100 mM phosphate for 14 h. The cultures were diluted 1:100 into the same medium and grown to an OD cm$^{-1}$ of approximately 2. The bacteria were resuspended in the same base medium and the OD cm$^{-1}$ was adjusted to 2. To categorize the species according to ‘suicidal’, ‘self-inhibiting’, ‘self-supporting’ and ‘neutral’ each species was grown in the same base medium once with a high buffer concentration (100 mM phosphate) and once with a low buffer concentration (10 mM phosphate). At the start of the experiment and after 24 h, the CFU was estimated. The pH was measured after 24 h with a pH microelectrode as described above.

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

Data availability. All data generated or analysed during this study are included in the Article (and its Supplementary Information).

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Author contributions
C.R., J.D. and J.G designed the research. J.D., C.R. and J.G. carried out the experiments and wrote the manuscript. We thank all members of the Gore lab for reading and discussing the results, and wrote the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41559-018-0535-1.
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Correspondence and requests for materials should be addressed to C.R. or J.G.
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Experimental design

1. Sample size
   Describe how sample size was determined.
   The experiments turned out to be very reproducible, thus the error between
different replicas and thus the error bars are very small. Moreover, are results and
conclusions are not based statistic methods but are of rather qualitative nature.
Because of these reasons we found at least 3 replicates for each quantitative
measurement sufficient.

2. Data exclusions
   Describe any data exclusions.
   We excluded the data of one replica in Supplemental Fig. 3. This data curve
showed a very strong optical density at time=0 and strong fluctuations of the signal
over time, which we assigned to the presence of contaminations from the
beginning of the measurement on (e.g. aggregated nanobeads or similar) and
removed that curve.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   We reproduces every measurement at least 2 times, by two independent people
(J.D and C.R.) with the same results. Often measurements were reproduced also
more often than 2 times.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   There was no randomization.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   We did not use blinding. The signals and the differences between the different
measurements were very clear, which did not make blinding necessary.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.
6. Statistical parameters
For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

|   | Confirmed |
|---|-----------|
| n/a |           |

- The exact sample size \((n)\) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- A statement indicating how many times each experiment was replicated
- The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
- A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- The test results (e.g. \(P\) values) given as exact values whenever possible and with confidence intervals noted
- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

7. Software
Policy information about availability of computer code

Describe the software used to analyze the data in this study.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

8. Materials and reagents
Policy information about availability of materials

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

The soil strains we used are available on request, other than that no unique material has been used.

9. Antibodies
Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

No antibodies were used.

10. Eukaryotic cell lines
a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used.

b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used.

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cell lines were used.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No eukaryotic cell lines were used.

Animals and human research participants
Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals
Provide details on animals and/or animal-derived materials used in the study.

No animals were used.
12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

No humans were used.