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Higher temperatures generically favour slower-growing bacterial species in multispecies communities

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Temperature is one of the fundamental environmental variables that determine the composition and function of microbial communities. However, a predictive understanding of how microbial communities respond to changes in temperature is lacking, partly because it is not obvious which aspects of microbial physiology determine whether a species could benefit from a change in the temperature. Here we incorporate how microbial growth rates change with temperature into a modified Lotka–Volterra competition model and predict that higher temperatures should—in general—favour the slower-growing species in a bacterial community. We experimentally confirm this prediction in pairwise cocultures assembled from a diverse set of species and show that these changes to pairwise outcomes with temperature are also predictive of changing outcomes in three-species communities, suggesting that our theory may be applicable to more-complex assemblages. Our results demonstrate that it is possible to predict how bacterial communities will shift with temperature knowing only the growth rates of the community members. These results provide a testable hypothesis for future studies of more-complex natural communities and we hope that this work will help to bridge the gap between ecological theory and the complex dynamics observed in metagenomic surveys.

Temperature fundamentally shapes the structure of microbial communities and determines whether an individual species will be able to survive and proliferate. Yet, outside of food science, little attention has been paid to how temperature alone influences the dynamics of microbial growth and competition, especially in complex communities. As changes in global temperatures alter the stability and structure of microbial ecosystems, it will be critical to understand how microbial communities react to warming environments and to determine whether there are general patterns that can be used to forecast these changes.

Although the specific effects of temperature on microbial communities remain unclear, many culture-independent microbial surveys have characterized communities that are affected by changing temperatures, either due to seasonal cycles or long-term warming. In the ocean, for example, there are profound cyclical changes to community structure that correlate with seasonal temperature variations[1–3]. Long-term changes in the biogeography of marine microorganisms may also be driven by temperature[4], as ecological niches shift toward the poles under a warming climate[5]. Changes in the structure of bacterial communities have also been observed in warming soils[6], with potentially important implications for the global carbon cycle[7]. Still, temperature is but one of many important variables in these studies, and it is difficult to disentangle temperature-dependent responses from those driven by changes in pH or nutrient availability. Furthermore, without extensive knowledge of the species within the community, it is not possible to determine what general factors lead them to be favoured or disfavoured by environmental alteration.

Few studies have specifically addressed the influence of temperature on competitive outcomes between microbial species. It has been shown that fluctuation in temperature can hold a plankton community away from equilibrium and enable coexistence when one species would drive the other extinct at a stable temperature[8,9]. Still, little is known about how changes to the steady-state temperature should alter the community state at equilibrium and whether there are general rules for how the temperature influences microbial competition.

In this paper, we develop a model that predicts how coculture outcomes between bacterial species with different growth rates should be affected by changes to the environmental temperature and that predicts that the species with the lower maximal growth rate should—in general—be favoured by increasing temperature. We also report experimental results that validate these predictions in two- and three-species communities. Together, these results suggest that there are general principles that govern how competitive outcomes are shaped by temperature, and that it may be possible to forecast which species should be favoured by a warming environment knowing only their growth rates.

Results

Experimental microbial communities are normally incubated at a fixed temperature. We aimed to determine how changing this incubation temperature affects the outcome of a microbial coculture in which the two species were known to stably coexist at the usual experimental temperature of 25 °C. We focused on two naturally co-occurring species isolated from soil (*Acinetobacter* strain 1 (Aci1) and *Pantoea* strain 1 (Pan1)), and followed a standard coculture methodology (see Methods) and three experimental temperatures: 16 °C, 25 °C and 30 °C. At each of these three temperatures, Aci1 is the faster-growing species and the difference in the growth rates of the two species increases together with the temperature (Fig. 1a). In accordance with this, we assumed that the slower-growing Pan1 would be favoured when decreasing the temperature and disfavoured when increasing the temperature, as its competitive ability would probably be hindered by a larger disparity in growth rate. Surprisingly, we observed the opposite and found that Pan1 becomes a stronger competitor at the higher temperature, with the coculture outcome

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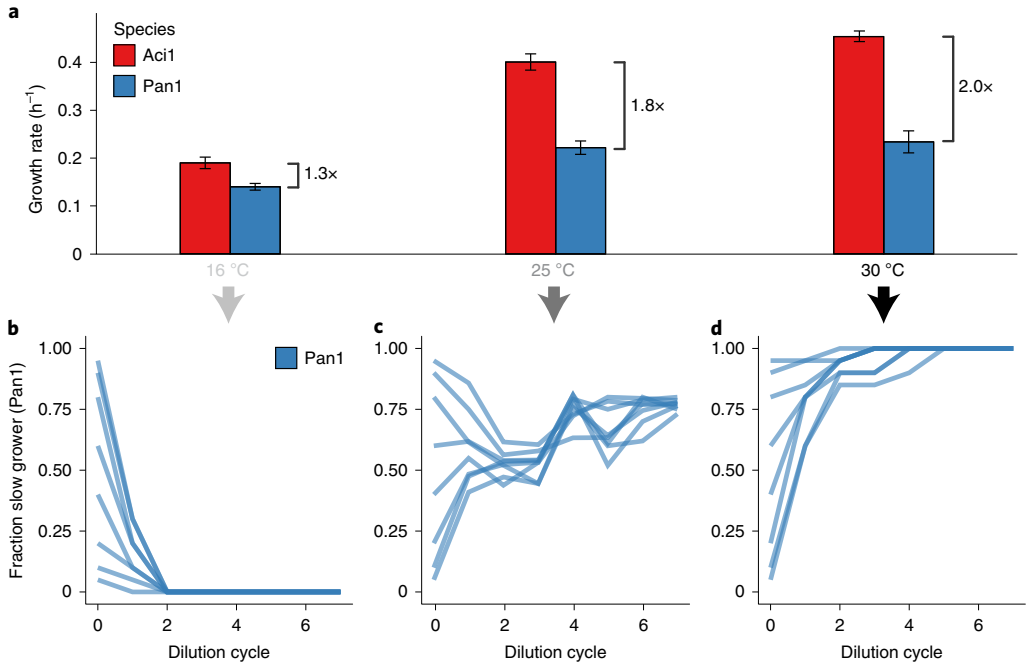


Fig. 1 | Increasing temperature favours the slower-growing bacterial species in a coculture, despite an increase in the difference in growth rates. The coculture outcomes of a faster-growing *Acinetobacter* species (Aci1) and a slower-growing *Pantoea* species (Pan1), both isolated from the same soil sample. **a**, Aci1 is the faster grower regardless of temperature and the difference in growth rates between the two species accelerates as temperature increases. Data are mean ± s.e.m. **b**–**d**, Increasing the temperature moves the community state at equilibrium from competitive exclusion by Aci1 at 16 °C (**b**) to coexistence at 25 °C (**c**) and eventually to Pan1 dominance at 30 °C (**d**), with the potentially counterintuitive result that the slower-growing species is favoured at higher temperatures even when that change increases the difference in growth rates.

shifting from Aci1 dominance at 16 °C (Fig. 1b) to coexistence at 25 °C (Fig. 1c) and finally to Pan1 dominance at 30 °C (Fig. 1d).

To explain this potentially counterintuitive result, we developed a model that expands on a previous study[10], who used a modified version of the Lotka–Volterra competition model to explain how increasing mortality favours faster-growing species. In addition to the growth rates, the Lotka–Volterra model requires knowledge of how the growth of the two species is inhibited by other cells of their own species compared with the presence of cells of the competing species. This inhibition is traditionally captured by a parameter (α) that relates the strength of interspecific (between-species) competition to intraspecific (within-species) competition (Fig. 2a). Competitive outcomes in the classic version of this model are determined entirely by these competition coefficients. However, many microbial communities experience mortality that is not driven by competition and that affects the entire community. Notably, this is true of all laboratory cultures, in which cells are removed from the community either continuously (for example, in a chemostat or turbidostat) or at discrete intervals (for example, in batch culture). It may also result from predation by bacterivores or from physical removal, such as in the case of gut microbiota. The formulation of the Lotka–Volterra model described above can therefore be made more realistic for microbial cocultures by the introduction of a community-wide mortality rate (δ). The introduction of this death rate to the model (Fig. 2b) has an important effect: it makes the competitive outcome dependent on the growth rates as well as the competition coefficients, such that when the death rate is absorbed the parameter α is reparametrized as

$$\hat{\alpha}_{\mathrm{fs}} = \alpha_{\mathrm{fs}} \frac{1 - \frac{\delta}{r_{\mathrm{s}}}}{1 - \frac{\delta}{r_{\mathrm{f}}}} \quad (1)$$

where α_{fs} is the inhibition of the faster-growing species by the slower-growing species without the death rate and $\hat{\alpha}_{\mathrm{fs}}$ is the inhibition with the death rate. Adding mortality to the model favours the faster-growing species[9] by increasing $\hat{\alpha}_{\mathrm{sf}}$ and decreasing $\hat{\alpha}_{\mathrm{fs}}$. Visually, this change to the α of the two species can be represented as a 45° arrow through the phase space of the competitive outcomes (Fig. 2c), pointing to the quadrant in which the faster grower wins. Mortality can reverse the competitive outcome if the slow grower would win without the mortality rate, passing first though a region of either coexistence or bistability. Notably, the arrow is made longer by higher death rates and shorter by higher growth rates. As bacterial growth rates are a function of temperature, this in turn introduces a temperature dependence to the competition and suggests that, at any given death rate, higher temperature should favour the slower-growing species by lessening the favour conferred to the fast grower by the added mortality.

To understand how temperature influences the growth rate of bacterial species, we used the model of Ratkowsky et al.[11]. This phenomenological model predicts a linear relationship between temperature and the square root of the growth rate of a species, such that the growth rate of any bacterial species can be modelled (so long as it is sufficiently below the optimum temperature (T_{Opt}) of a species) as a function of two parameters: the slope of the presumed linear relationship (b) and the x-axis intercept of that line (T_0) (Fig. 2d). For any pair of species in which there is a consistent fast grower (that is, the faster grower has a lower T_0 and a higher b than the slower grower), including the Ratkowsky model into the competition model and taking the derivative of α_{fs} with respect to temperature reveals the prediction that the slow grower is always favoured by an increase in temperature. Notably, this is true even if the difference in growth rates between the two species increases with temperature. The prediction is largely generalizable to any temperature range in which there is a consistent faster-growing

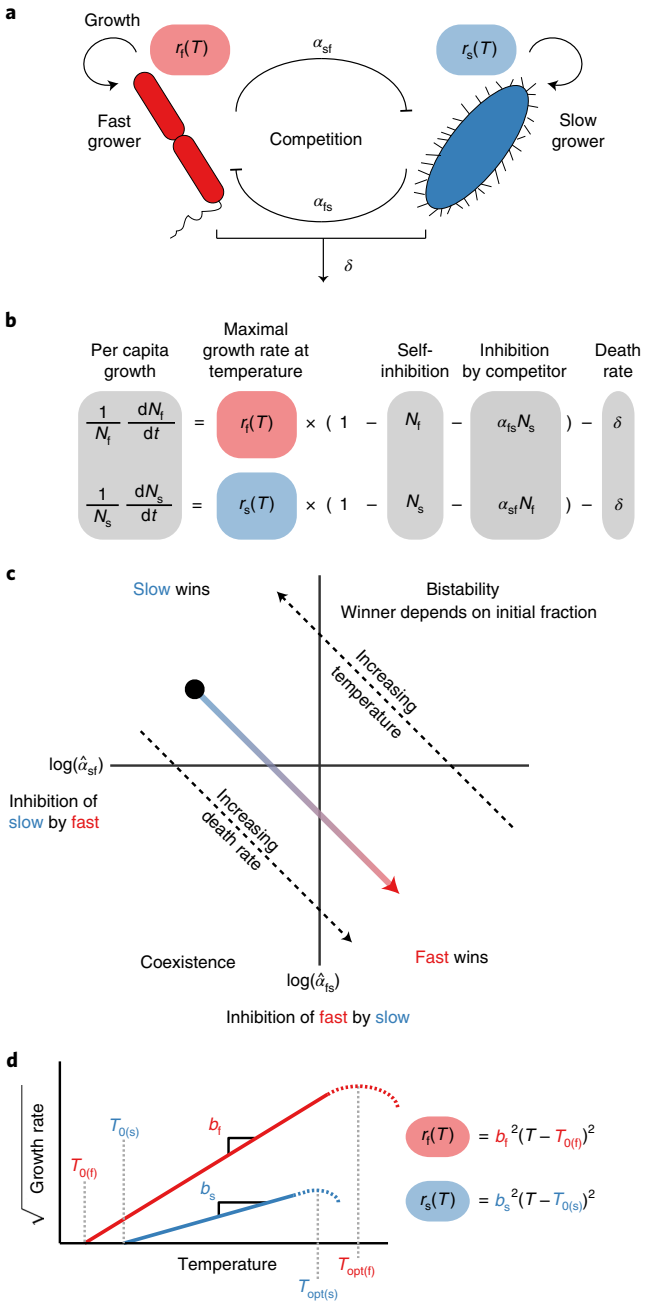


Fig. 2 | A simple model predicts that the slower-growing species in a coculture should generally be favoured by increasing temperature. **a**, The Lotka–Volterra competition models are parameterized by the growth rates of the two species and their competition coefficients (α), which relate between-species inhibition to within-species inhibition. A community-wide death rate (δ) can also be added to the model. **b**, The full Lotka–Volterra competition equations, with added death rate. **c**, With no death rate, competition outcomes are determined exclusively by the competition coefficients and do not depend on the growth rates (black dot). The introduction of a death rate can alter the competitive outcome by effectively increasing the $\log(\alpha)$ of the fast grower and decreasing the $\log(\alpha)$ of the slow grower by the same amount, resulting in a 45° movement through phase space. This arrow becomes longer as the death rate increases and shorter as temperature (and accordingly growth rates) increases, indicating that for a given death rate, the slower-growing species should be favoured by an increase in temperature. **d**, The growth rates of microorganisms when sufficiently below T_{Opt} are a simple function of temperature that can be modelled with two parameters: the slope of the square root of the growth rate against temperature (b) and the minimum growth temperature (T_0).

species and slower-growing species, even if their growth-rate rankings flip far enough outside this temperature range (Supplementary Information). It is also generalizable to non-competitive interactions such as mutualism and parasitism (Extended Data Fig. 1). There are practical limits to this change to α: if the slow grower is already dominating at low temperatures then increasing the temperature will not lead to a qualitative change in the outcome. Additionally, this model only holds when the temperature is below the optima of the species and growth rates increase together with the increases in temperature. Notably, the term 'optimum temperature' is used throughout this paper to mean the temperature at which a species reaches its maximum growth rate in monoculture, and not the temperature at which it does best in competition. This theory suggests that it is possible to alter the competitive outcome by changing the temperature and to predict which species should benefit so long as the growth rates are known.

As a test of this theory, we chose a collection of 13 bacterial strains with variable growth rates (Fig. 3a). This group comprised six strains from the American Type Culture Collection (ATCC) and seven naturally co-occurring strains isolated from soil. To fit the Ratkowsky model, we measured the growth rates of each strain at a minimum of four temperatures using a time-to-threshold approach (Fig. 3b, Extended Data Fig. 2 and Methods). Both model parameters had a wide range, with T_0 ranging from −14 °C to 4 °C (mean = −3 °C, s.d. = 5 °C) and b ranging from 0.012 to 0.031 (mean = 0.024, s.d. = 0.005). Notably, these two values were highly correlated (Pearson's $\rho = 0.96$; Fig. 3b), suggesting that species that are capable of growing at lower temperatures (lower T_0) are less able to increase their growth rates as the temperature increases (lower b). This correlation has been reported previously[12], although—to our knowledge—without any mechanistic explanation. It follows from the high correlation between b and T_0 that the curves that represent the growth-rate responses to temperature of different species (Fig. 3b) are likely to intersect, such that which of the species is called the 'fast grower' and 'slow grower' may not be consistent across our range of temperatures. However, in 39 of the possible 78 pairs of species (50%), there was a consistent fast-growing and slow-growing species across the range of experimental temperatures (16–30 °C) (Fig. 3c). We carried out 38 of these 39 pairwise cocultures; we did not coculture Pan1 and Pan2 because the morphologies of their colonies are difficult to visually differentiate. For a subset of these cocultures, we varied the death rate as well as the temperature to explore how these two variables interact to shape competitive landscapes (Extended Data Fig. 3).

When these coculture outcomes are visualized as a heat map (Fig. 3d), we observe a clear shift from a dominance of the fast grower at 16 °C to coexistence or dominance of the slow grower in most species pairs. Plotting the changes in the percentage of the occurrence of the slow grower for the two temperature shifts (Fig. 3e) reveals that almost all transitions are in line with our theory. Of the 73 transitions that do not include a bistable outcome, 46 (63%) resulted in an increase in the percentage of the slow grower in accordance with our theory, 23 (32%) led to no shift in the percentage of the slow grower and only 4 (5%) resulted in a decrease in the percentage of the slow grower, in contrast to our theoretical predictions. In two of the four pairwise transitions that were not predicted by the model (*Enterobacter aerogenes* (EA)–*Serratia marcescens* (SM) and Aci2–*Pseudomonas veronii* (PV), both for the transition from 16 °C to 25 °C), the fast grower dominated the community at 16 °C when its initial fraction was 90% but coexistence was observed when its initial fraction was 50% or 10%, suggesting that the community may not have reached an equilibrium within the 7-d experiment. In the third pair (*Pseudomonas putida* (PP)–Pan2), we always observed coexistence at both 25 °C and 30 °C, but with very high variance between replicates (0.1%–0.8% slow grower at 25 °C and 0%–0.8% slow grower at 30 °C), suggesting either experimental

error or a high degree of stochasticity in this particular interaction. Finally, the transition of the fourth pair (*Pseudomonas chlororaphis* (PCH)–PV) from 16 °C to 25 °C consistently showed a switch from coexistence to dominance of the fast grower, suggesting that another temperature-dependent factor influenced the community in a direction that is in contrast to our theory. To determine whether our experimental results were biased by the limited phylogenetic diversity (all 13 species are members of the class Gammaproteobacteria) or by the specific environmental conditions of our protocol, we further tested our theory in a separate set of competitions in Lysogeny broth (LB) medium with oxygen-permeable covers (Extended Data Fig. 4). These competitions were drawn from a set of six species belonging to four different bacterial phyla (the Proteobacteira, Actinobacteria, Firmicutes and Bacteroidetes) and further validated the theory that the slower-growing species should be favoured by higher incubation temperatures.

Given the accuracy of the model in predicting pairwise outcomes, we wanted to explore how temperature influences more-complex communities. Previous work in this group had developed a simple predictive algorithm for inferring microbial community assembly from pairwise interactions[13], which predicts that any species that is outcompeted in pairwise competition will not survive in any complex community that includes the other species in the pair. This suggests that a change in temperature that shifts a pairwise interaction from competitive exclusion to coexistence could have broad implications for other species in the community, potentially resulting in cascading effects. The reverse is also possible: changes in the temperature might shift a competitive outcome from coexistence to exclusion, decreasing the diversity of the community or allowing a species that was excluded by the newly outcompeted species to invade. We chose four trios of strains to test whether the changes that we observed in the pairwise dynamics propagated to a three-species community. For each trio, we competed each pair of species in the trio from two initial species fractions and the full trio from four initial starting fractions (Fig. 4a). We predicted that the community assembly rules should hold regardless of temperature and that increasing the temperature should shift the equilibrium state away from the fastest grower and towards the slowest grower.

We found that the community assembly rules were generally highly accurate: the standardized Euclidean distance of the prediction had a mean of 0.11, a s.d. of 0.17 and was 0 in more than half of the cases (32; 52%) (Fig. 4b). The error in our community assembly predictions was predominately driven by a single trio (Aci2–*Pseudomonas* strain 2 (Pseu2)–Pan1) for which coexistence was predicted for all three species (Extended Data Fig. 5a). At higher temperatures, the slow grower (Pan1) either reached a greater fraction than predicted (25 °C) or won the competition outright (30 °C). Notably, this error is driven by the assembly rules and not by our theory for how competitive outcomes should change with temperature, which apply only to pairwise interactions and were not violated in any pair of species that was included in the three-species competitions.

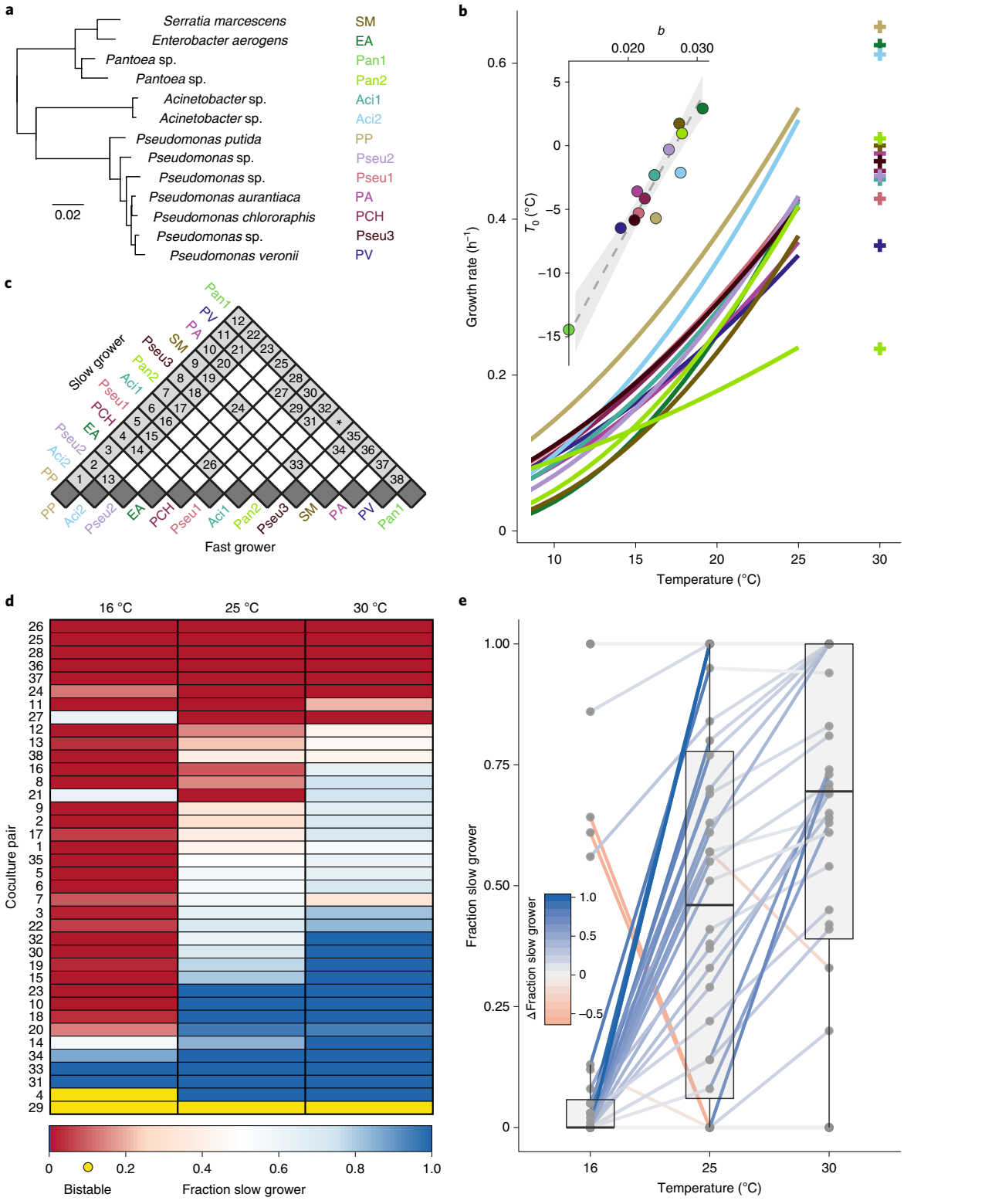
Here, we focus on the PP–PCH–SM trio, in which the assembly rules predict a shift from bistable dynamics between PP and PCH at low temperature, to coexistence between PCH and SM at intermediate temperature and ultimately to dominance by SM at high temperature (Fig. 4c). In this trio, there is a consistent fast (PP) and slow (SM) grower across the full range of temperatures (Fig. 4d), and the predictions from the pairwise dynamics are consistent with a movement in the species fractions at equilibrium away from the fast grower and towards the slow grower as the temperature increases. This is in fact what we observed in our experiment: in almost all cases the equilibrium result was qualitatively the same as that predicted by the assembly rules. We also observed interesting dynamics in the PP–PV–Pan1 trio (Extended Data Fig. 5b). In this case, PV was always excluded, but the species it was excluded by changed from PP to Pan1 as temperature increased. On the basis of the pairwise dynamics, it is possible that there is a temperature between 25 °C and 30 °C at which PV should have been able to persist, highlighting how even very slight changes in temperature can alter the diversity of a microbial community.

Discussion

All microbial communities are structured by interactions between their constituent species and between those species and the abiotic environment. As microorganisms compete for space and resources they have a number of tools at their disposal in addition to the ability to grow faster[14], including the production of secondary metabolites that are toxic to their competitors (antibiotics), contact-dependent inhibition or antagonistic environmental alteration—for example, through pH modification[15]. Temperature has the ability to influence each of these mechanisms, for example, by varying the secondary metabolites produced by the community members[16,17], manipulating the ability of community members to withstand the metabolites of other species[18] or changing the pH range at which a species can grow[19]. Temperature may also have a role in determining the nutritional requirements of different species, potentially altering the nature of their ecological interactions and upsetting competitive hierarchies[20,21]. This complex set of interacting variables might suggest that predicting the effect of temperature on the competitive outcomes between microbial species requires a potentially intractable knowledge of each species in the community and the interactions between them. However, we demonstrate here that we can obtain a substantial amount of predictive accuracy without any knowledge of the mechanisms that underpin those interactions by instead focusing exclusively on growth rates.

Fig. 3 | Theoretical predictions that slower-growing species should be favoured by increasing temperature are validated in a wide array of experimental cocultures between a diverse set of species. a, We tested our hypothesis that the slower-growing species should be favoured by increasing temperature in two-species communities drawn from 13 bacterial strains. Strains that are classified to the species level were obtained from the ATCC and strains that are classified only to the genus level were isolated from a single soil sample and identified by sequencing the 16S ribosomal subunit. Branch length of the phylogeny, based on full 16S sequences, corresponds to the number of substitutions per base pair. *Enterobacter aerogens* is also known as *Klebsiella aerogenes*. **b**, We measured the growth rates for each strain at a minimum of four temperatures to fit the Ratkowsky model for the range of 8 °C to 25 °C. The growth rates at 30 °C, the temperature at which the model may no longer hold, were measured directly. Line colours match the label colour for each strain shown in **a**. Inset: the strong correlation (Pearson $\rho = 0.96$) between the two parameters of the Ratkowsky model. **c**, Of the 78 possible species pairs, 39 pairs (50%) had one strain that was consistently the faster grower across the range of experimental temperatures (highlighted in grey). We cocultured 38 of these pairs at 16 °C, 25 °C and 30 °C following the experimental protocol of Fig. 1. *We did not coculture Pan1 and Pan2 because their colony morphologies are difficult to differentiate. **d**, Heat map of coculture outcomes after a 7-day dilution cycle. Values are an average of at least four separate cocultures comprising three different initial strain ratios (90% fast grower, 50% fast grower and 10% fast grower). Numbers designate species pairs as in **c**. **e**, The competitive outcomes at the three experimental temperatures. Black lines indicate the median, lower and upper box boundaries correspond to the first and third quartiles, and whiskers extend to the largest and smallest values within 1.5× the interquartile range. Points indicate the outcomes of individual coculture pairs and pairs are connected by lines, which are coloured by the change in the mean equilibrium percentage of the fast grower. The two pairs for which we observe a bistable outcome are not included in the plot.

This surprising simplicity may result from the density dependence of each of these competitive mechanisms: the greater the gap between the growth rate and death rate of a species, the more the population of that species will be able to alter the environment in a manner favourable to itself. Even a very strong competitor will be rendered ineffectual if it is unable to reach a sufficient density. For example, a slower-growing strain that relies on antibiotic production as a competitive mechanism may not be able to produce a minimum inhibitory concentration if its growth rate is barely higher than the death rate. However, such density-dependent effects are not an essential requirement of the model. The prediction that a slow grower will benefit from increasing temperature remains true even in the case of non-interacting species ($\alpha = 0$). The ratio of carrying capacities is reparametrized by an added death rate:



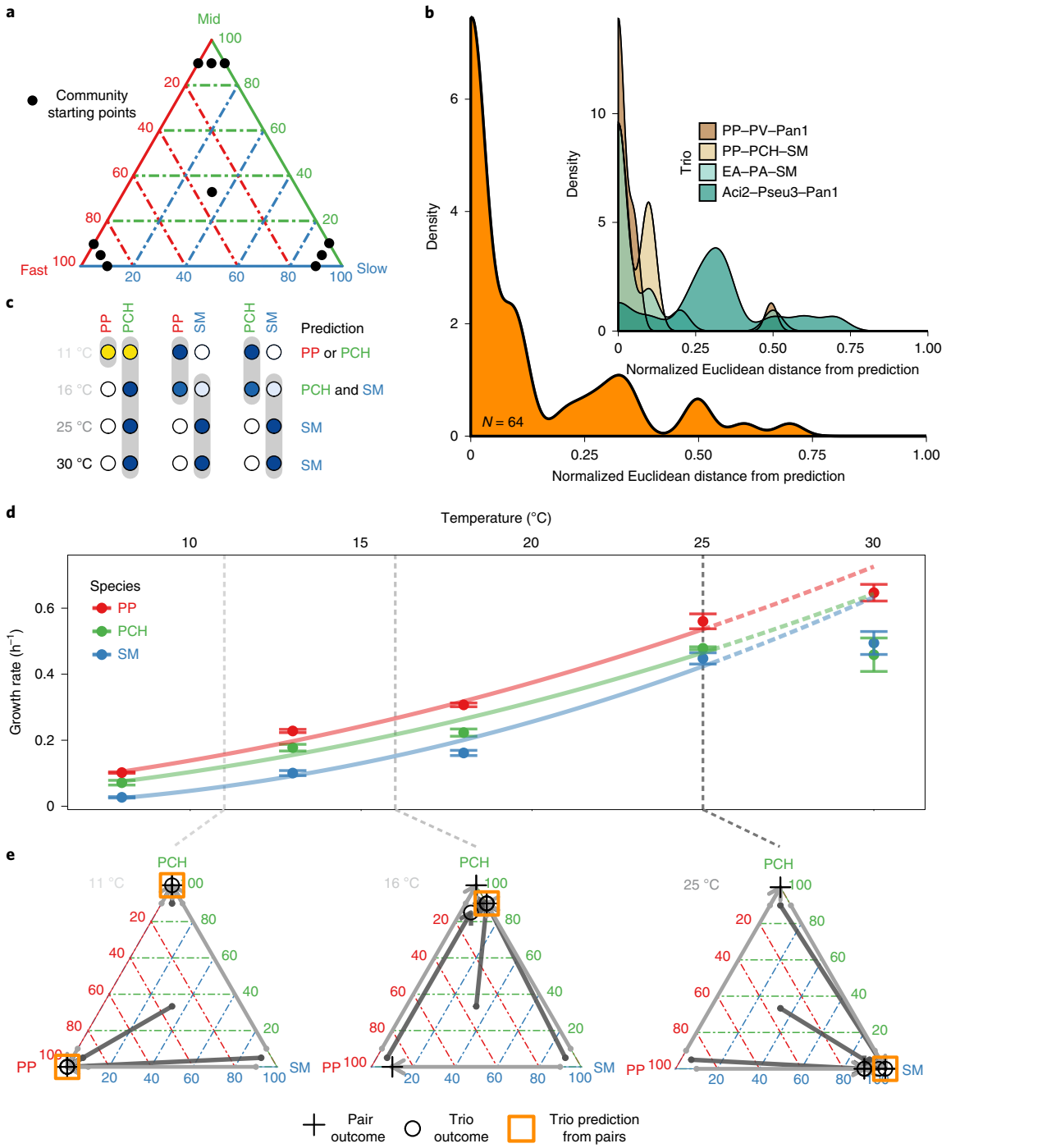


Fig. 4 | Shifts in pairwise competitive outcomes with temperature enable the prediction of the shifts observed in a three-species community. **a**, To test the predictive accuracy of pairwise dynamics for a three-species community, we determined the equilibrium outcome of each pair in the trio for two initial starting points and the equilibrium outcome of the full trio for four starting points. **b**, We calculated the Euclidean distance (normalized to the maximum possible distance) between the predicted and observed equilibrium states. The outer density plot shows the distribution of distances across all four trios, while the inner plot splits the distribution by trio. **c**, We used the assembly rules of a previously published study[12] to estimate the community state at equilibrium from the pairwise dynamics of each species in the trio. In this example, the pairwise dynamics predict a bistable outcome between PP and PCH at 11 °C, coexistence between PCH and SM at 16 °C and dominance by SM at 25 °C and 30 °C. **d**, The PP–PCH–SM trio has a consistent growth-rate hierarchy regardless of temperature. **e**, The experiment validates both the predicted movement away from dominance of the fast grower about the ternary plot and the predictive accuracy of the assembly rules. Note that the 30 °C outcome is not shown because it is identical to the 25 °C treatment.

$$\frac{\hat{K}_s}{\hat{K}_f} = \frac{K_s}{K_f} \times \frac{1-\frac{\delta}{r_s}}{1-\frac{\delta}{r_f}} \quad (2)$$

The term by which the ratio is multiplied is the same as that in equation (1) and increases with temperature when the growth rates increase according to the Ratkowsky model. In the absence of interactions, the slow grower thus occupies a relatively larger

niche as temperature increases; adding interactions only amplifies this effect.

Although our experimental results agree with the prediction of the Lotka–Volterra model, which was modified to include the Ratkowsky model, this confirmation does not necessarily mean that the model is accurate, and there could be a simpler explanation. We tested a number of competing hypotheses for why competitive outcomes may change with increasing temperature, starting with whether our results might be driven by an overall decrease in the difference in the growth rates between the fast and slow grower (ΔGR) with increasing temperature. However, we observe the opposite (Extended Data Fig. 6a): at 16 °C, the only cases in which the slower-growing species won or persisted in the competition were those for which the ΔGR was very small, leading to a pronounced negative correlation between ΔGR and the fraction of the slow grower. This negative correlation was substantially decreased at 25 °C and switched signs at 30 °C, indicating that large differences in growth rate do not hinder the competitive abilities of slow growers at higher temperatures so long as the species are still below their temperature optimum. A specific example of a pair in which increasing the temperature favours the slow grower despite an accompanying increase in ΔGR is shown in Fig. 1.

We also tested whether changes to the competitive outcome may be driven by differences in the proximity to the optimum temperatures of the two species. Although we do not know the exact optimum temperatures of our species, we do have some insights into their temperature preferences. PV was the only species in our study that was unable to grow when incubated at 37 °C, indicating that its maximum and optimum temperatures are relatively low, whereas EA was the only species in our study that was capable of growth at 42 °C, suggesting that its maximum and optimum temperatures are relatively high. Both EA and PV were included in competition experiments in which they were the slower-growing species, and the prediction held regardless of their temperature preferences. This suggests that in a competition between two species, increasing the temperature generally favours the slower grower, regardless of which species has a higher optimum or maximum temperature. We also found no significant correlation between the competitive outcomes and the differences in the T_0 values of the two species at any temperature (Extended Data Fig. 6b). Although T_0 is not an exact estimate for the minimum growth temperature, it seems that larger differences in T_0 (ΔT_0) may be a reasonable proxy for larger differences in the optimal temperature. We did not find a significant relationship between ΔT_0 and competitive outcome at any temperature; this, coupled with our observations about EA and PV, leads us to believe that differences in the optimum temperature of the two competing species are not a major driver of the competitive outcomes.

We also used experiments with spent medium to gain insight into the competitive mechanisms in our cocultures (Extended Data Fig. 7). With the exception of a few clear cases of inhibition (PP by the slower-growing PCH and Pseu1, and *Pseudomonas aurantiaca* (PA) by the faster-growing PCH), we were largely unable to clarify the competitive mechanism, although we did observe a few cases of non-competitive interactions, including in the pair (Aci1–Pan1) shown in Fig. 1. In this case, the interaction appears to be parasitic (Pan1 grows twice as fast in the spent medium of Aci1 as it does in its own spent medium, whereas Aci1 grows half as fast in the spent medium of Pan1 as it does in its own spent medium), highlighting how our theory holds across diverse types of interactions. Other species, especially PV, PCH and Pseu1, had very similar growth rates across all spent media. Notably, these species tended to be strong competitors at higher temperatures, especially PCH, which always dominated the community at 30 °C (across fives competitions).

Finally, we explored whether alternative models of microbial competition resulted in the same theoretical prediction that the slower-growing species should generally be favoured by increasing temperature, which is always the case when there is a consistent slow grower in the Lotka–Volterra model (Supplementary Information). We find that this prediction is still true when considering a linear resource-concentration model with a single resource, but that it is not always true in a one resource Monod model or in a model that incorporates multiple resources (Supplementary Information).

Here, we demonstrate a potentially unifying predictive ability that only requires knowledge of a single variable: the maximal growth rate of each species. Although encouraging, these results are based on simple two- or three-species communities, drawn from a small species pool, in a tightly controlled laboratory environment. Still, this theory and preliminary experimental work provides a testable hypothesis for future studies of more-complex natural communities and helps to bridge the gap between ecological theory and the complex dynamics observed in metagenomic studies.

Methods

Species and medium. We used two sets of bacterial species in this study: seven naturally co-occurring taxa isolated from soil and six strains that were obtained from the ATCC. The soil isolates were obtained by vortexing a small amount of soil taken from an urban park into phosphate-buffered saline, followed by plating onto LB agar. Colonies were chosen to be visually differentiable from all other strains in the study and capable of growing in the defined medium described below. The taxonomic identity of the soil isolates was determined by sequencing the V4–V5 16S hypervariable region, and the seven isolates were found to comprise representatives from three bacterial genera: *Acinetobacter* (Aci1 and Aci2), *Pantoea* (Pan1 and Pan2) and *Pseudomonas* (Pseu1, Pseu2 and Pseu3). The six species obtained from ATCC were *E. aerogenes* (also known as *Klebsiella aerogenes*, ATCC 13048), *P. aurantiaca* (ATCC 33663), *P. chlororaphis* (ATCC 9446), *P. putida* (ATCC 12633), *P. veronii* (ATCC 700474) and *S. marcescens* (ATCC 13880). All 13 strains are members of the bacterial class Gammaproteobacteria.

All coculture experiments in this study were carried out in S minimal medium supplemented with glucose (0.2%) and ammonium chloride. The medium contained 100 mM sodium chloride, 5.7 mM dipotassium phosphate, 44.1 mM monopotassium phosphate, 5 mg l^{-1} cholesterol, 10 mM potassium citrate pH 6 (1 mM citric acid monohydrate and 10 mM tripotassium citrate monohydrate), 3 mM calcium chloride, 3 mM magnesium sulfate, trace-metal solution (0.05 mM disodium EDTA, 0.02 mM iron sulfate heptahydrate, 0.01 mM manganese chloride tetrahydrate, 0.01 mM zinc sulfate heptahydrate and 0.01 mM copper sulfate pentahydrate), 0.93 mM ammonium chloride and 10 mM glucose.

Growth rate model. To fit the Ratkowsky model for each strain, we calculated their growth rate at a minimum of four temperatures. We used a time-to-threshold approach to estimate growth rates, in which monocultures with known initial optical density (OD at 600 nm) were spot checked every few hours. These growth-rate experiments were carried out as follows: frozen stocks of the desired species were streaked out on a nutrient agar Petri dish and, after incubation at room temperature for around 48 h, a single colony was picked and transferred into 5 ml of 1× LB broth and grown overnight. Then, 35 µl of this LB culture was inoculated into 5 ml of S medium and grown for about 24 h. The OD of the S medium culture was measured and the background OD (measured as the OD of the same volume of sterile S medium in the same type of 96-well plate) was subtracted to estimate the population density. A $\log_{10}$ serial dilution of the monoculture was carried out on a 300-µl 96-well plate (Falcon) so that each strain was diluted to an OD of between 10^{-1} and 10^{-6} that of the overnight culture. The OD of each of these diluted cultures was checked periodically, the background OD was subtracted and the growth rate was calculated as $\log(OD_T/OD_{T=0})/T$ where $OD_{T=0}$ is the initial OD of the diluted culture and OD_T is the OD at time T (measured in hours from the initial time point). To ensure that the cultures were still in their exponential phase of growth, the growth rate was only calculated for measurements with $OD_T < 0.15$ and all growth rate estimates were based on a minimum of five measurements. This method of measuring the growth rate implicitly incorporates lag time, as strains with a longer lag times will take longer to reach a given OD than another species with the same exponential growth rate but a shorter lag time.

Coculture experiments. Frozen stocks of the competing species were streaked out on nutrient agar Petri dishes and, after incubation at room temperature for around 48 h, a single colony of each species was picked and transferred into its own 50-ml Falcon tube containing 5 ml of 1× LB broth. Monocultures were grown overnight at room temperature and 35 µl of this LB culture was then inoculated into 5 ml S medium and grown for around 24 h at room temperature. The monocultures of each species were then OD-standardized and the monocultures were mixed together with the desired proportions. In the two-species experiments, the cocultures started from three initial community states: 90% fast grower–10% slow grower, an equal split and 10% fast grower–90% slow grower. In the three-species experiments, the competitions started from four initial community states: three

90%–5%–5% splits, each with a different species in the majority, and one with an even split of 33.3% of each species. All competition experiments were carried out in 300 µl 96-well plates (Falcon). The initial plate was made by adding 160 µl S medium, 20 µl 2% glucose and 20 µl of a 1:10 dilution of the appropriate mixed cultures to each well. The plate was then incubated, wrapped in Parafilm and without shaking, for 24 h at the desired temperature. Each day, for seven cycles, the plate from the previous day was serially diluted into new S medium so that each well held 180 µl of a 1:100 dilution of the mixed culture and 20 µl of 2% glucose was added before incubation for another 24 h. At the end of the competition cycle, the cultures were spotted onto nutrient agar after dilution in phosphate-buffered saline and colonies were counted by visual inspection to determine the equilibrium fraction of the species.

Spent-medium experiments. We used a spent-medium assay to help to clarify the mechanisms that our set of species used to compete against each other. We grew 24 h cultures of the eight species most commonly used in pairwise competitions (PCH, PP, PV, SM, Aci1, Aci2, Pan1 and Pseu1), and sterilized the spent medium through two consecutive filtrations with 0.22-µm Millipore Steriflip vacuum-driven filters. Spent medium was then plated to verify its sterility. This spent medium was used in place of water in modified S medium, with all other reagents added back in the same concentrations as usual. We grew 11 of our 13 species in each of these eight spent media for a period of around 48 h and calculated the maximal growth rate for each species in each medium type using the time-to-threshold protocol described above.

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

Data availability

The data are publically available through the FigShare digital repository[22].

Code availability

All code for data analysis is available from the first author by request.

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Author contributions

All authors designed the study. S.L. carried out the experiments with assistance from C.I.A. S.L. analysed the data. C.I.A. analysed the Lotka–Volterra and other models, and wrote the Supplementary Information. S.L. wrote the manuscript, and all authors edited and approved it.

Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41559-020-1126-5.

Supplementary information is available for this paper at https://doi.org/10.1038/s41559-020-1126-5.

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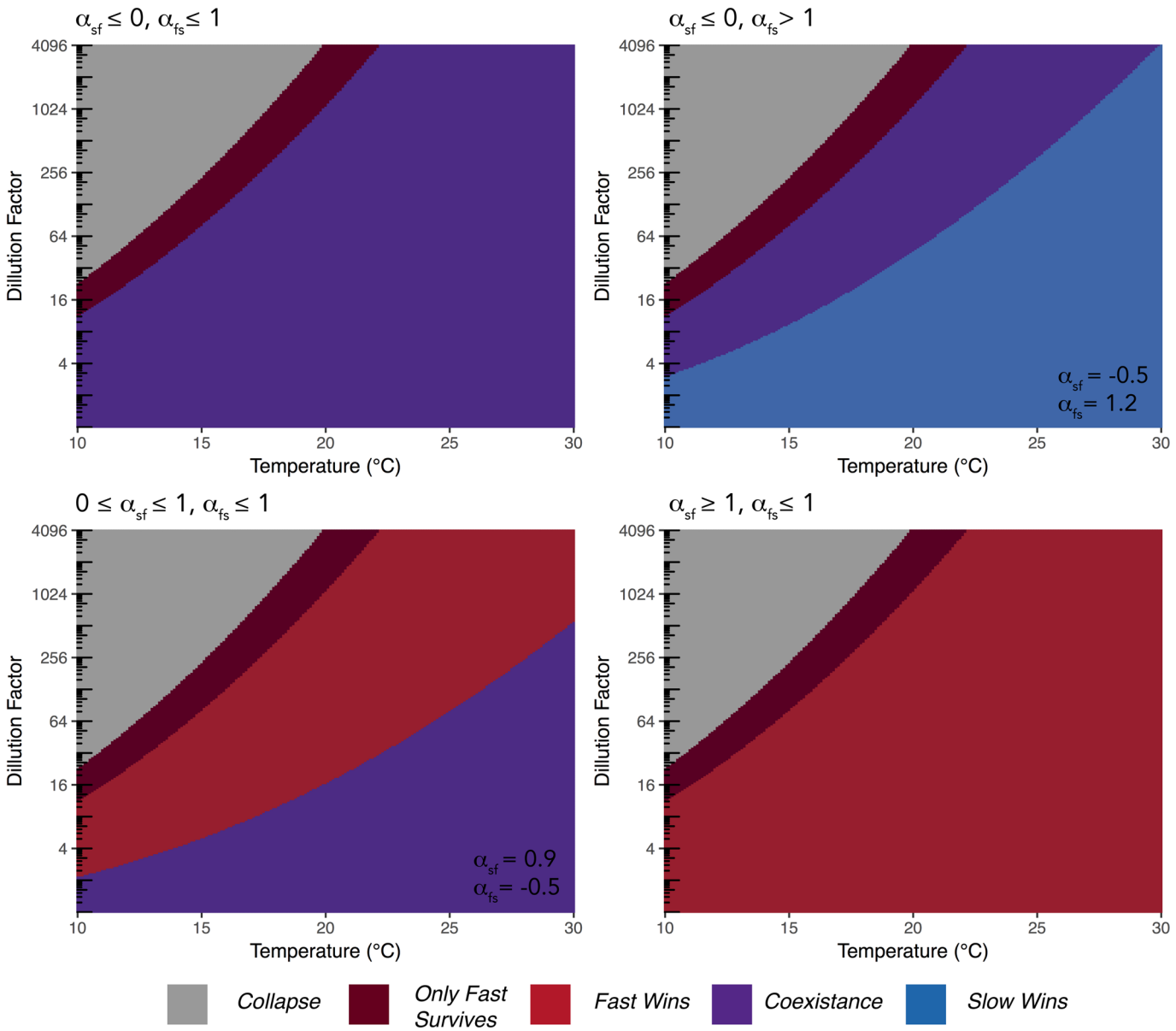
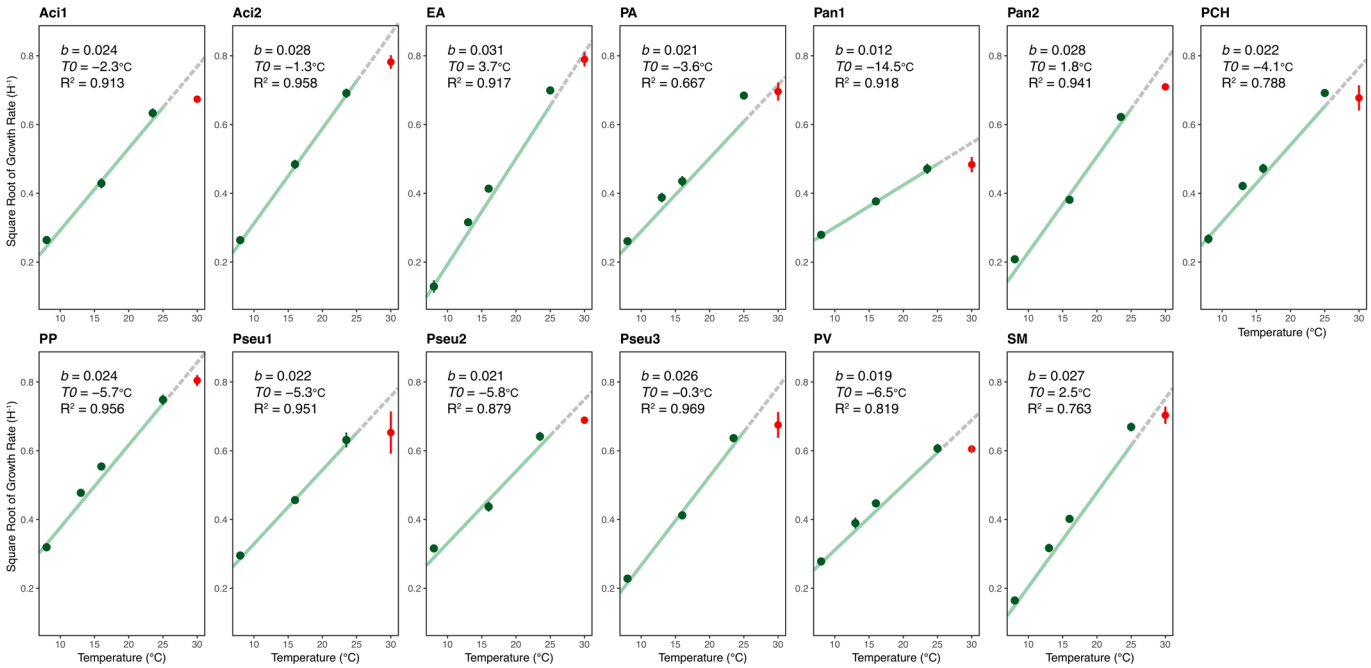
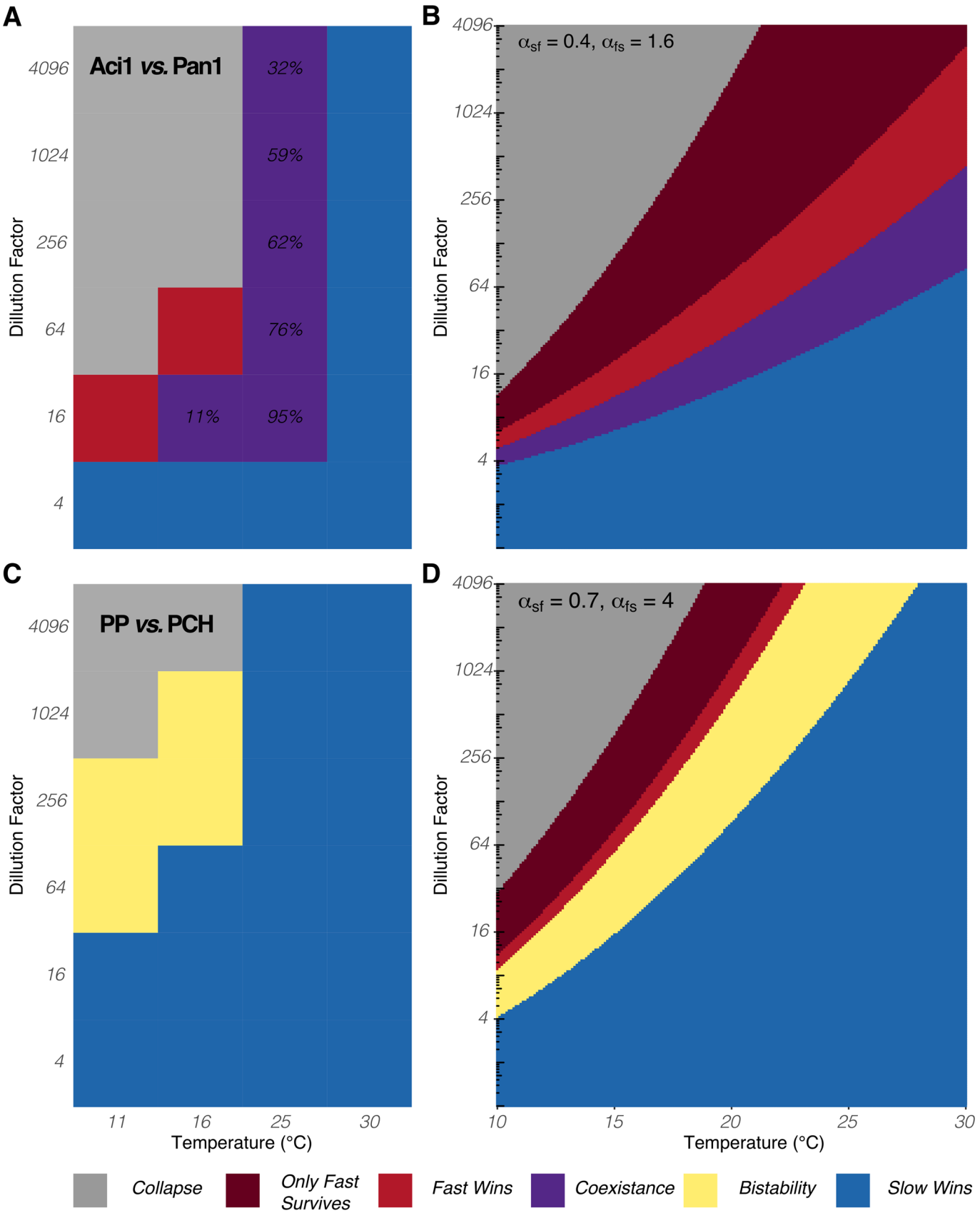


Extended Data Fig. 1 | Predicted phase spaces of coculture outcomes with noncompetitive interactions. The model is based on a hypothetical faster-growing species with the parameters b = 0.0230 and T0 = −5.7 and slower growing species with b = 0.0250 and T0 = −4.1. In cases where there are multiple non-trivial qualitative outcomes, the specific α's are indicated at the bottom left of the plot. If no specific α's are indicated, the qualitative phase space is identical for all α's in the title range. In mutualist pairs, coexistence is always expected in any region of phase space where the slower-growing species is able to survive the imposed death rate, such that decreasing temperature can only move the outcome into a trivial fast-grower victory ($r_s < \delta < r_f$) or community collapse. Parasitic interactions, however, depend on the α's. If the fast grower assists the slow grower ($\alpha_{sf} < 0$) but the slow grower harms the fast grower ($\alpha_{fs} > 0$), we expect coexistence in all non-trivial regions of phase space ($r_s > \delta$) if $\alpha_{fs} < 1$ and a transition from coexistence to slow-grower dominance with increasing temperature if $\alpha_{fs} > 1$. If the slow grower assists the fast grower ($\alpha_{fs} < 0$) but is hindered by the fast grower ($\alpha_{sf} > 0$), we expect either consistent fast grower dominance if $\alpha_{sf} > 1$ or a transition from fast-grower dominance to coexistence with increasing temperature if $0 < \alpha_{sf} < 1$.

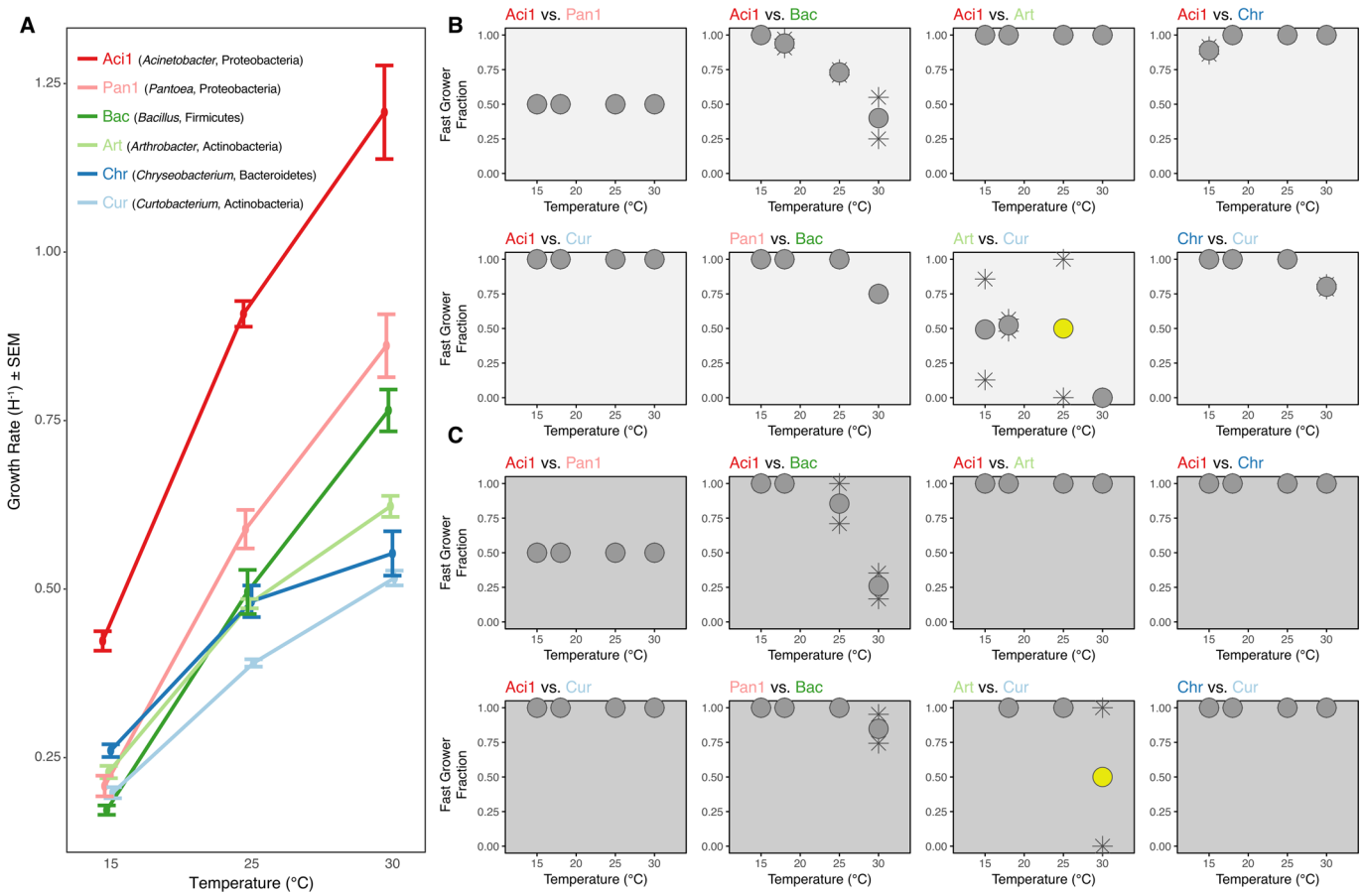


Extended Data Fig. 2 | Fits of the Ratkowsky model to the 13 bacterial strains in this study. The Ratkowsky model predicts a linear relationship between temperature and the square root of the growth rate. We used linear regression (light green line) on our growth rate estimates below 30 °C (dark green circles, SEM indicated by bars) to estimate *b* (the slope of the regression) and *T0* (the x-intercept of the regression). The 30 °C growth rates ± SEM are plotted in red. The R^2 of the regression is also provided.

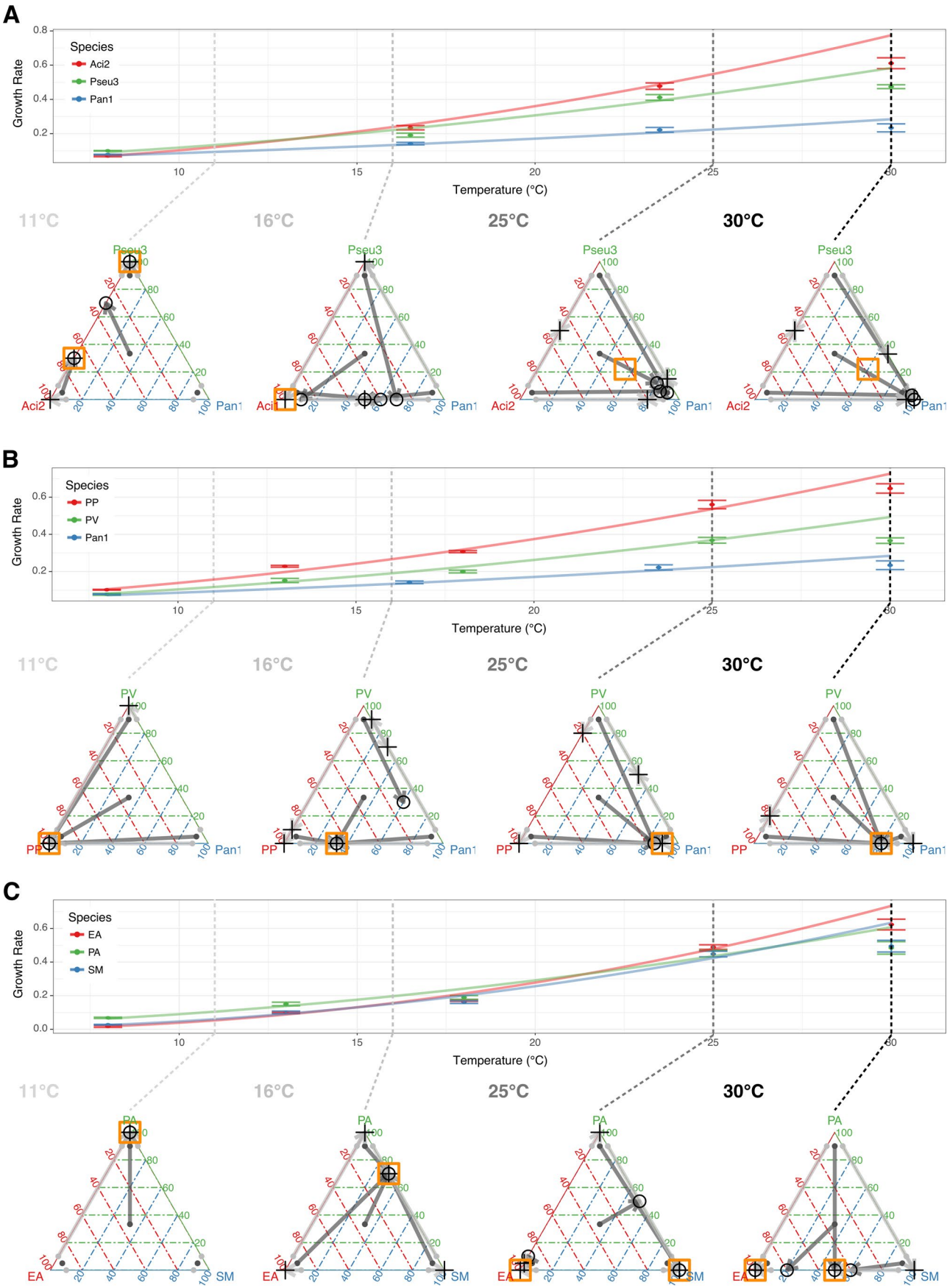


Extended Data Fig. 3 | See next page for caption.

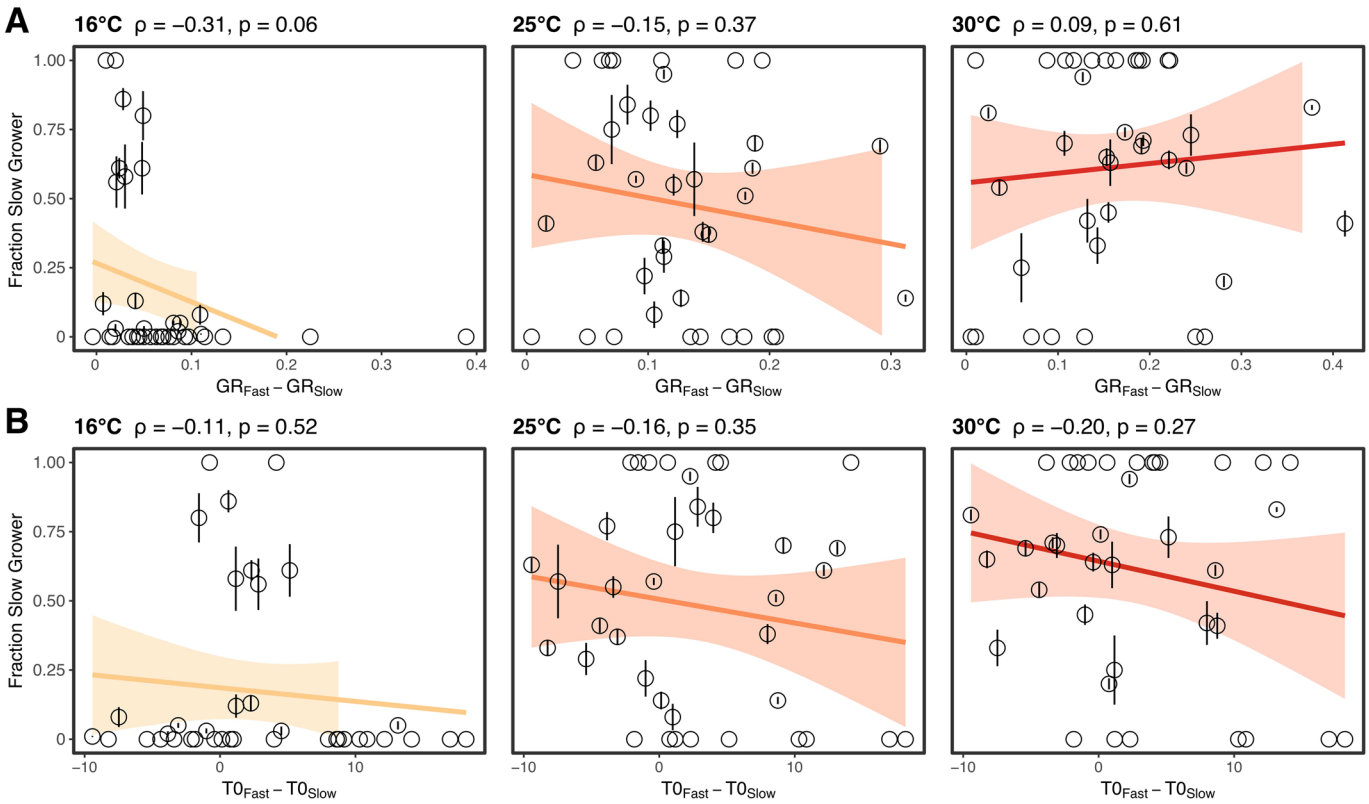
Extended Data Fig. 3 | Phase diagrams of the interaction between temperature and dilution factor for two sets of coculture outcomes. (**a**) We varied temperature (4 levels) and dilution factor (6 levels) in a factorial design to understand how these two variables interact to shape competitive landscapes. Here, we have the qualitative outcomes for the Aci1/Pan1 competition for each combination of variables. In cases of coexistence, the percentage of the final community comprised by the slow-grower is indicated. (**b**) Phase diagram of the model predictions for the Aci1/Pan1 competition, parameterized with the measured b and T0 values, and with α's (indicated at top left) estimated to best match the experimental outcome. (**c**, **d**): As in **a**, **b**, but for the PP/PCH competition.



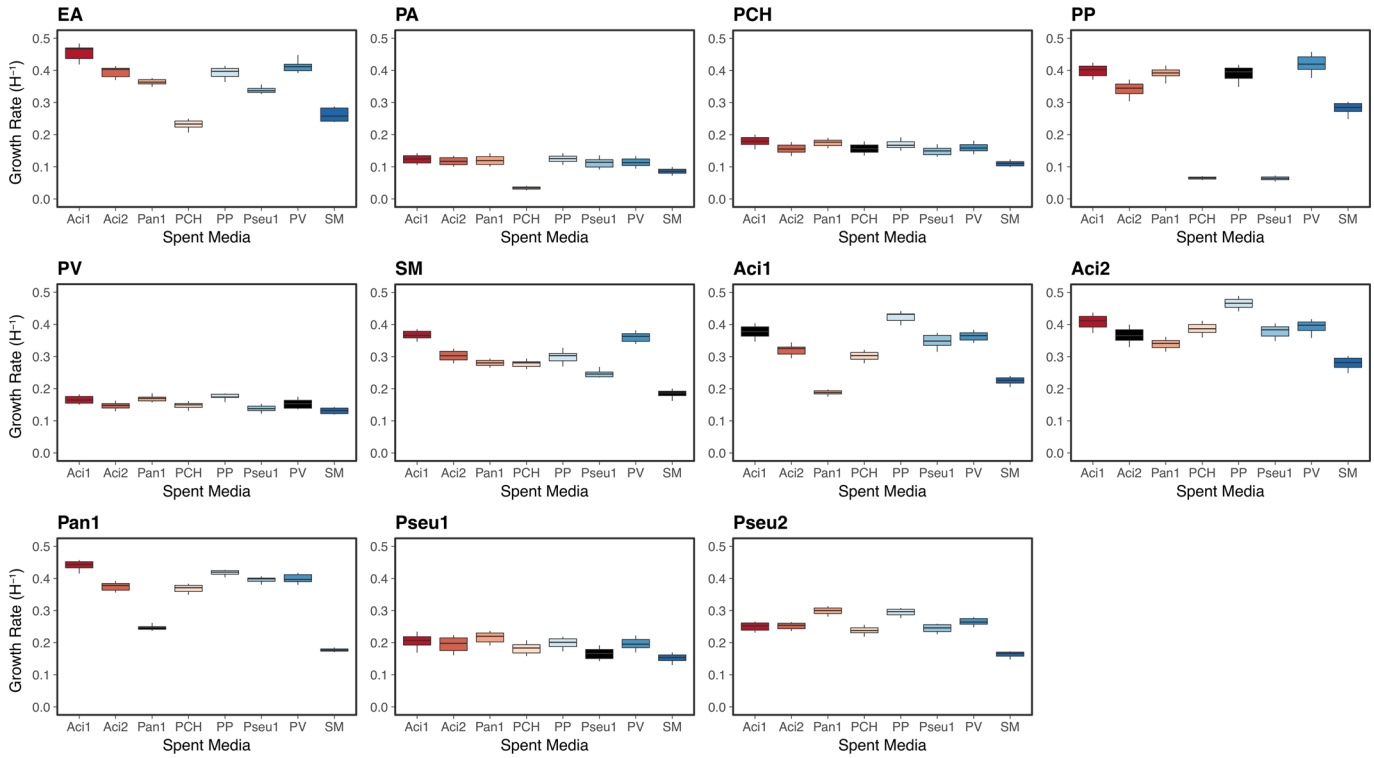
Extended Data Fig. 4 | Competitions between a phylogenetically diverse set of species in a complex media further validate the prediction that higher temperatures favor slower-growing bacterial species. (**a**) Growth rate measurements for the 6 bacterial species in 100% LB media with an oxygen permeable cover. Error bars represent the standard error of the mean. (**b**) Equilibrium species fraction after 7 cycles of a 1/100 daily dilution. We competed the 8 pairs of species where there is a consistent fast grower across the range of experimental temperatures. The y-axis represents the fraction of the faster growing species. Competitions were started at two initial conditions: 90% fast grower and 10% fast grower. Asterisks represent the outcome of an individual condition and circles represent the average outcome across the two initial conditions. Cases where the outcome is bistable are indicated in yellow. (**c**) As in **b** except with a 1/1000 daily dilution. Note that in both **b** and **c** we were unable to count individual colonies in the Aci1/Pan1 competition because of very high cell density. Aci1 and Pan1 always coexisted at approximately equal fractions.



Extended Data Fig. 5 | Coculture outcomes in the three species communities not plotted in Fig. 4. Growth rate plots are formatted as in Fig. 4d and ternary plots are formatted as in Fig. 4e.



Extended Data Fig. 6 | Relationship between coculture outcome and difference in growth rate parameters between the faster-grower and slower-grower. Correlation values are Pearson correlation, error bars on the y-axis correspond to the SEM, and trend lines are a linear regression with the 95% confidence interval. (**a**) Correlation between growth rate difference and coculture outcome by temperature. (**b**) Correlation between T0 difference and coculture outcome by temperature.



Extended Data Fig. 7 | Maximal growth rates for 11 species growing in the spent media of the 8 most common species in our set of competition experiments. Black lines indicate the median, lower and upper box boundaries correspond to the first and third quartiles, and whiskers extend to the largest and smallest values within 1.5 times the inter-quartile range. Colors indicate the origin of the spent media, and black coloration indicates that the species is growing in its own spent media.

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Study description	We varied the incubation temperature of replicated two- or three-species communities to assess how temperature influences the competitive outcomes between these species. For a subset of the communities, we also varied the dilution factor in a factorial design with temperature.
Research sample	Samples were two- or three-species communities drawn from a pool of 13 bacterial species. Data was collected as the percent of the community comprised by the different species (as inferred through colony counts) at the end of the 7 day experiment.
Sampling strategy	We cocultured every pair of species in which there was a consistent faster- and slower-growing species across our temperature range. To screen for bistability, each community was started at three initial species ratios: 10% fast/90% slow, 50% fast/50% slow, and 90% fast/ 10% slow.
Data collection	At the end of each 7 day dilution cycle, the community was diluted to between 10^-4 and 10^-6 its initial concentration, and 10uL of the diluted community was plated onto agar. Colonies, which could be visually differentiated, were counted ~48 hours after plating (always by the first author), and the ratio of the colonies was recorded.
Timing and spatial scale	All coculture experiments lasted for 7 dilution cycles.
Data exclusions	No data were excluded from analysis, with the exception of some early results from communities which we later realized did not have a consistent faster-growing species. From the 39 potential pairs of species were there is a consistent faster-growing species, we did not coculture one pair (Pan1/Pan2) because it was too difficult to distinguish the colony morphologies.
Reproducibility	Each community was replicated at least 4 times, with different initial fractions of the species. In most (but not all) cases, these replicates were split across at least two separate experiments so that the results would include biological replicates in addition to technical repliates.
Randomization	NA
Blinding	NA

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