Short-term relative invader growth rate predicts long-term equilibrium proportion in a stable, coexisting microbial community

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Abstract

The ability of species to mutually invade from rare is the defining measure of species coexistence. However, it is unknown whether invasion growth rates predict any characteristic of long-term community dynamics. Here, we use a model five-species microbial community to investigate the link between short-term growth rate and long-term relative abundance. We manipulated diversity and tested the ability of species to coexist in different combinations. Across all diversity levels and species combinations, populations re-established from rare in 71 of 75 combinations and all combinations were stable in long-term culture. Moreover, short-term relative invader growth rate was positively associated with long-term equilibrium proportion, despite large variation in interactions between species and communities. This finding was confirmed using a modelling approach and suggests that the short-term invasion growth rate can predict long-term relative abundance within that community.

Introduction

Determining whether species can coexist is a central aim of ecology (Chesson 2000; Barabás *et al.* 2018). To this end, modern coexistence theory focuses primarily on a single metric: the mutual invasibility criterion that measures the recovery of species from low density (Chesson 2000, 2018; Grainger *et al.* 2019). If all species in a community recover from low density when the other species are at a higher density, then the community members can theoretically coexist (Chesson 2000; Barabás *et al.* 2018; Ellner *et al.* 2019; Grainger *et al.* 2019). Such short-term invasion assays are designed to demonstrate negative frequency dependence, with previous work generally looking at whether, for each species, low density growth rate is positive (Grainger *et al.* 2019) or relative invader growth rate (low density growth rate relative to the growth rate of the other higher-density species) is greater than 1 (Hodgson *et al.* 2002; Chesson 2018). When this occurs, coexistence is theoretically possible, but the importance of the value of relative invader growth rate has received less attention (Pande *et al.* 2020).

Does the value of relative invader growth tell us something beyond the qualitative outcome of whether or not a species combination can coexist? Previous theoretical work suggests that it might, with a model based around modern coexistence theory finding a positive relationship between invader growth and species abundance (Chisholm & Muller-Landau 2011). However, there are, to our knowledge, no experimental tests of this relationship. An analogous relationship has been suggested in macroecology: the niche centrality hypothesis posits that equilibrium abundances should be higher in environments where low density growth rate is higher (Martínez-Meyer *et al.* 2013; Dallas & Hastings 2018; Holt 2019; Osorio-Olvera *et al.* 2019). Evidence in support of this hypothesis is however equivocal: correlational patterns both support (Martínez-Meyer *et al.* 2013) and refute (Dallas & Hastings 2018) the niche centrality hypothesis across animal and plant communities. Recent theory has highlighted why this macroecological relationship may not be ubiquitous, with spatial and temporal variation

60 and metapopulation dynamics potentially causing this relationship to break down (Holt 2019;
61 Osorio-Olvera *et al.* 2019).

62 Any relationship between relative invader growth rate and equilibrium density may be
63 weakened when moving from species pairs, as used most commonly in modern coexistence
64 theory, to diverse communities. This problem is both practical and theoretical. Practically, re-
65 establishing from rare is measured by removing species *n* from a community or invading
66 species *n* from a much lower density. However, following its removal or reduction in density,
67 the community may destabilise or convert to an alternative stable state (Chesson 2018). Such
68 community-wide changes may make it impossible to study the invasion of a species into the
69 community (*n*-1). For example, in rock-paper-scissors (intransitive) competition, each species
70 decreases the abundance of another that in turn decreases the abundance of another in an
71 interaction chain (Kerr *et al.* 2002; Lankau & Strauss 2007; Levine *et al.* 2017). Thus, if one
72 species was removed the system would break down. From a theoretical standpoint, the relative
73 invader growth rate and equilibrium abundance of species *n* are context dependent and are
74 determined not only by the species' own intrinsic growth parameters but also by those of all
75 the other species in the community (Chisholm & Muller-Landau 2011; Barabás *et al.* 2016).
76 Greater species diversity increases the possibility of increased context dependency, which may
77 weaken the link between relative invader growth and equilibrium abundance. The link could
78 be further weakened by the presence of complex indirect interactions at greater levels of
79 diversity (Allesina & Levine 2011; Bairey *et al.* 2016; Levine *et al.* 2017) that can alter
80 community dynamics in ways not predicted from models based on pairwise interactions alone
81 (Friedman *et al.* 2017; Mayfield & Stouffer 2017; Letten & Stouffer 2019; Sanchez-Gorostiaga
82 *et al.* 2019).

83 Here, we experimentally investigate whether invader growth rate predicts equilibrium
84 density (relative abundance). To understand this, a highly stable community is needed where

species can coexist at multiple levels of diversity and in different combinations of species. We allowed a community to naturally (dis)assemble and then tested the stability of the resulting 5 species community. In doing so, we measured relative invader growth rate and equilibrium density across all species combinations and levels of diversity. We complemented our empirical work with simulations based on Lotka-Volterra competition to explore the generality of our findings.

Methods

Overview of model microbial community

We created a model microbial community consisting of 5 species that (dis)assembled and coexisted for approximately 85 generations. Briefly, this community was formed by inoculating 46 bacterial strains – belonging to 27 genera (Table S1) – that were isolated from an experimental compost community (Hesse *et al.* 2018) into growth medium (1/64 Tryptic Soy Broth (TSB) diluted with demineralised H_2O) and incubated for 13 weeks. After this time period, the 48 communities converged on a similar composition of ~5 dominant species. We selected a single replicate of these to explore its stability across levels of diversity and investigate the link between relative invader growth rate and equilibrium abundance. This community consisted of five species, *Achromobacter* sp., *Ochrobactrum* sp., *Pseudomonas* sp., *Stenotrophomas* sp., and *Variovorax* sp. Crucially, each of these species could be identified by their colony morphology (Table S2; confirmed by sequencing 16S rDNA of multiple colonies of each morphotype (Padfield *et al.* in prep)), allowing us to easily, and affordably, manipulate diversity and track each species in the community. A single clone of each member of this community was picked and grown in monoculture for two days in 6mL of growth media, shaking, in 25mL glass vials with loosened plastic lids at 28ºC to achieve high cell densities.

109 These samples were then cryogenically frozen at -80ºC in glycerol (final concentration: 25%).
110 These clones were then used to recreate the communities for subsequent experiments.
111
112 *Measuring coexistence, relative invader growth rate and equilibrium proportion*
113 To assess whether diversity influenced species coexistence, we conducted invasion-from-rare
114 assays, where each species was inoculated from rare in every species by diversity combination.
115 In total, this yielded six replicates of 75 treatments. Species were grown from the freezer stocks
116 to achieve high cell densities as described above. Cell density (colony- forming units [CFU])
117 was estimated from optical density (600nm wavelength; OD_{600}) using a species-specific
118 calibration curve (Table S3 for equations) and normalised to 10^5 CFU μL^{-1}. Focal species were
119 invaded at 100-fold lower density (1.6µL) than that of the resident community (160µL in total).
120 When there was more than one resident species, resident communities were created from equal
121 ratios of each species. Cultures were incubated for one week, after which samples were
122 cryogenically frozen at -80ºC in glycerol. Species abundances (CFU mL^{-1}) were estimated by
123 plating cultures onto KB (King's medium B) agar and incubating for two days at 28°C.
124 To test whether diversity impacts long-term coexistence and persistence, each species
125 was also inoculated at equal density in every possible community combination, from
126 monoculture to five-species. Species monocultures were prepared as described earlier, with 20
127 µL of each species inoculated (total inoculum varied across diversity levels) into fresh
128 microcosms for each community combination. Twelve replicates of the 31 unique community
129 combinations were then incubated for either 1, 2, 3 or 4 weeks (three replicates each) without
130 resource replenishment. This enables us to measure equilibrium abundances and allows time
131 time for extinctions to occur and to measure the effect of resource scarcity and secondary
132 metabolites for cultures left for 3 or 4 weeks. At the end of each incubation period, individual

133 species and total community abundance (CFU mL^{-1}) were estimated using methods described
134 above.
135
136 *Statistical analyses*
137 All analyses were carried out in *R* using the *tidyverse* packages (Wickham *et al.* 2019). Mixed
138 effects models and contrasts were performed using the *R* packages *lme4* (Bates *et al.* 2014) and
139 *emmeans* (Lenth 2018), and model selection was done using likelihood ratio tests. To analyse
140 invasion-from-rare assays, relative invader growth rate of the focal species was calculated as
141 the ratio of estimated Malthusian parameters (m), m_{focal}:$m_{\text{community}}$ where $m_{\text{community}}$ is the total
142 density change of all the other populations combined. $m = ln(N_1 / N_0)/t$ where N_1 is the final
143 density, N_0 is starting density, and t is the assay time (7 days)(Lenski *et al.* 1991). We performed
144 independent one-sample t-tests on each combination of focal species by resident community
145 combination, with the null-hypothesis being that the mean relative invader growth rate equals
146 1. A relative invader growth rate above 1 would indicate that the invader had a faster growth
147 rate than the resident community, demonstrating negative frequency dependence. This resulted
148 in 75 different statistical tests; P values were adjusted using the false discovery rate (*fdr*)
149 method (Benjamini & Hochberg 1995).
150 To analyse the long-term coexistence assays, the equilibrium abundance (log_{10}-
151 transformed) of each focal species was calculated at the end of each week. Abundances from
152 week 1 were removed as it was visually clear that equilibrium density had not been reached for
153 most species. We used a linear mixed effects model to analyse whether equilibrium species
154 abundance (log_{10}-transformed CFUs mL^{-1}) changed over different levels of diversity (as a
155 discrete variable) and focal species as potentially interacting predictors. A random effect was
156 added to account for the non-independence of multiple measurements of the same focal species
157 in the same community combination. We also investigated whether total community

158 productivity changed with diversity, where the response variable was the $\log_{10}$-transformed
159 mean (averaged across all weeks) total community abundance (CFUs mL^{-1}) of each community
160 combination. We treated diversity as a continuous predictor as there was only one unique
161 combination of the 5 species community.

162 To assess the relationship between relative invader growth rate and equilibrium
163 proportion, we combined the data from the short- and long-term coexistence experiments. For
164 the invasion assays, relative invader growth rate was averaged (by taking the mean) across
165 replicates for each focal species in each community combination. For the long-term
166 experiments, equilibrium proportion for each species was calculated as N_{focal}/N_{total}. As
167 equilibrium abundance was not reached after 1 week of growth for most species in the long-
168 term experiments, we were confident that our invasion-from-rare assays measured growth rate,
169 not carrying capacity. However, we checked that the relative difference between the abundance
170 of the invading species reached at the end of the invasion-from-rare assay and equilibrium
171 density ($N_{invasion}/N_{long\text{-}term}$) did not correlate with equilibrium proportion (Pearson's correlation:
172 t = -0.31, df = 73, P = 0.75) and relative invader growth rate (t = 1.92, df = 73, P = 0.06). We
173 further checked whether length of incubation impacted equilibrium proportion using a
174 generalised linear mixed effects model with a binomial error structure: equilibrium proportion
175 was the response variable, length of incubation as the predictor with a random effect accounting
176 for the non-independence of data points across focal species and community combination.
177 There was no impact of incubation time on equilibrium proportion (ANOVA between model
178 with and without time: χ^2_1= 0, P = 0.99), so we averaged (by taking the mean) across different
179 incubation times for each focal species in each community combination. This left us with a
180 single measurement of equilibrium proportion and relative invader growth rate for each species
181 in each community combination with more than one species (n=75). We used a binomial
182 model, where mean equilibrium proportion of the focal species was the response variable, with

183 mean relative invader growth rate as a predictor, and focal species included as a potential
184 interacting factor.

185 To investigate how species interactions changed with diversity, we characterised the
186 species interactions of the communities using the long-term coexistence experiments. We
187 modified a multiplicative framework originally developed to help quantify higher order
188 antibiotic interactions on *Escherichia coli* (Tekin *et al.* 2018a). However, as our assays cannot
189 determine whether the presence of a third species changes competitor density (an interaction
190 chain) or changes per capita competitive effects (higher order interaction), our method just
191 calculates indirect interactions generally. This method overcomes the issues of the additive
192 model when single species have large impacts on fitness, such that the additive expectation can
193 never be achieved (i.e. a population size < 0)(Tekin *et al.* 2017, 2018b, a). Species interactions
194 (e.g. effect of species *2* on species *1*) were calculated as the abundance of species *1* in the
195 presence of species *2* relative to that of species *1* grown in monoculture (no interspecific
196 interactions): $w_{1|2} = N_{1|2}/N_1$. If *w* is greater than 1, then the interaction is positive, whereas
197 a $w < 1$ demonstrates competitive interactions, with a value of 0 demonstrating competitive
198 exclusion.

199 For each community combination with more than two species (e.g. species *1, 2, 3*), the
200 predicted effect on the focal species, $\widehat{w}_{1|23}$, is the product of the independent pairwise
201 interactions: $\widehat{w}_{1|23} = w_{1|2} \times w_{1|3}$. The indirect interaction (*ii*) of any community combination
202 on focal species *1*, $ii_{1|23}$, was calculated as the deviation of the observed abundance relative to
203 monoculture, $w_{1|23}$, from that predicted from pairwise interactions: $ii_{1|23} = \widehat{w}_{1|23} - w_{1|23}$. A
204 value of 0 would mean that there is no indirect interaction and pairwise species interactions
205 can predict dynamics in that community combination. To get a broad perspective of the nature
206 of species interactions, we calculated the average (the mean of ~9 replicates) pairwise species
207 interaction or indirect interaction for each focal species in each community combination. To

208 explore how average pairwise interactions and average indirect interactions change between
209 species, we ran separate linear models with focal species as a predictor and average interaction
210 strength as the response variable. To look at the significance of average interaction strength,
211 we compared the 95% CI to 1 (for pairwise interactions) and 0 (for indirect interactions).

212 We tested the ability of pairwise interactions to predict abundance in more complex
213 communities (>2 species) by calculating the R^2 of the relationship between predicted *vs.*
214 observed abundance relative to monoculture of the focal species across all community
215 combinations containing >2 species. To do this, we calculated the residual variation (residual
216 sum of squares/$RSS = \sum(x - \hat{x})^2$) and total variation in species interactions (total sums of
217 squares/$TSS = \sum(x - \bar{x})^2$) and calculated R^2 as (1 - (RSS/TSS)). A value close to 1 indicates
218 a model with close to zero error, and a value close to zero indicates a model very close to the
219 baseline of an intercept only model (mean species interaction). When R^2 is negative, it means
220 that the model is worse than simply predicting the mean species interaction.

221

222 *Simulating the relationship between short- and long-term coexistence*

223 To examine whether any relationship between relative invader growth rate and equilibrium
224 proportion was general or specific to our simple model community, we created a simple
225 continuous Lotka-Volterra two-species competition model for species *1* and *2* which calculates
226 the rate of change *dN/dt* at a given abundance, *N* (Volterra 1927; Lotka 1956).

227
$$\frac{dN_1}{dt} = N_1.r_1.(1 - \frac{N_1 + \alpha_{12}.N_2}{K_1})$$
$$\frac{dN_2}{dt} = N_2.r_2.(1 - \frac{N_2 + \alpha_{21}.N_1}{K_2}) \quad (1)$$

228 where r_1 and r_2 are the growth rates of species *1* and *2*, and K_1 and K_2 are their respective
229 carrying capacities. The additional terms $\boldsymbol{\alpha}_{12}$ and $\boldsymbol{\alpha}_{21}$ account for the species interactions, where
230 $\boldsymbol{\alpha}_{12}$ represents the effect species *2* has on the population of species *1*. We simulated 5000

231 random, normally distributed values for r_1 and r_2 (mean [μ]=0.8, standard deviation [$\boldsymbol{\sigma}$]=0.2),
232 K_1 and K_2 (μ=650, $\boldsymbol{\sigma}$=100) and $\boldsymbol{\alpha}_{12}$ and $\boldsymbol{\alpha}_{21}$ (μ=0.7, $\boldsymbol{\sigma}$=0.2). Sampled parameters were chosen
233 to promote coexistence as values of $\boldsymbol{\alpha} < 1$ (interspecific competition < intraspecific
234 competition).

235 We ran three models on each set of parameters using the function '*ode*' from the *R*
236 package '*deSolve*' (Soetaert *et al.* 2010). The only difference between models was the initial
237 abundance, *N*, of the species: one model started with 50 individuals of both species, the other
238 models simulated invasion-from-rare assays with 100 and 1 individuals of each species
239 respectively. Only converged models were kept, with convergence defined as when the slope
240 of abundance ~ time for each species in the last 20% of time points was <0.0001. Simulations
241 were filtered to only keep simulations where coexistence occurred (e.g. relative abundance of
242 one of the species did not equal 1 or 0). Equilibrium proportion of each species calculated as
243 N/N_{tot} where *N* of each species was averaged (by taking the mean) over the last 20% of the time
244 points. Relative invader growth rate was calculated as the ratio of Malthusian parameters
245 (where N_0 was always the initial density) and was averaged (by taking the mean) over the first
246 15 time points. So, for each of the three models, there was a single estimate for relative invader
247 growth rate and equilibrium proportion for each species. A binomial regression was then used
248 to examine the relationship between short-term relative invader growth rate and long-term
249 equilibrium proportion.

250

251 **Results**

252 *Short- and long-term coexistence*

253 We determined whether all species were key for coexistence by invading each species from
254 rare in all species combinations. Species could invade, irrespective of resident community
255 composition or diversity, in 71 of the 75 combinations (one-sample t-test: $P_{adj} < 0.05$, Figure

256 1). Of the non-significant combinations, three were when *Achromobacter* sp. was the invading
257 species (including when it was invading into the other 4 species together; Figure 1a), the other
258 when *Stenotrophomonas* sp. was invading into a community of *Achromobacter* sp., *Variovorax*
259 sp. and *Ochrobactrum* sp. However, although not significant, mean estimates of relative
260 invader growth rate were still above 1 (see Table S3), but there was considerable variation in
261 relative invader growth rate, ranging from a minimum of 1.12 to a maximum of 4.34.

262 In the long-term coexistence trials, across cultures left for 2, 3 or 4 weeks, no species
263 went extinct in any of the community combinations across any levels of diversity (Figure 2;
264 Table S4). This result suggests that our invasion-from-rare assays successfully predicted
265 coexistence in the longer term (based on extinctions). When considering equilibrium
266 abundance of individual species, there was no significant interaction between species and
267 diversity levels (ANOVA comparing models with and without an interaction between species
268 and diversity: χ^2_{16}= 11.72, P = 0.76), but the individual effects were significantly associated.
269 Equilibrium abundance differed between species (ANOVA comparing models with and
270 without species as a predictor: χ^2_4= 98.61, P < 0.001), with *Stenotrophomonas sp* reaching the
271 highest abundance (mean = $10^{8.91}$ CFUs mL^{-1}, 95%CI: $10^{8.81}$-$10^{9.02}$; Figure 2d) and
272 *Pseudomonas sp* reaching the lowest equilibrium abundance (mean = $10^{8.11}$ CFUs mL^{-1},
273 95%CI: $10^{8.01}$-$10^{8.22}$ CFUs mL^{-1}, Figure 2c). Diversity levels were also significantly associated
274 with equilibrium abundance (ANOVA comparing models with and without diversity as a
275 predictor: χ^2_4= 37.12, P < 0.001), declining from monoculture (mean = $10^{8.7}$ CFUs mL^{-1},
276 95%CI: $10^{8.53}$-$10^{8.88}$ CFUs mL^{-1}) to the 5 species community (mean = $10^{8.23}$ CFUs mL^{-1},
277 95%CI: $10^{8.06}$-$10^{8.4}$ CFUs mL^{-1}). The marginal R^2 of the best species abundance model was
278 0.60, but the R^2 rose to 0.80 when accounting for the random effects, indicating that there is
279 substantial variation in equilibrium abundance between species combinations within the same
280 diversity level.

The decline in species abundance with increasing diversity meant that there was no relationship between community productivity and diversity (Figure 2f; ANOVA comparing models with and without diversity as a continuous predictor: $F_{1,30}$ = 2.81, P = 0.10). The community appeared to have a fixed carrying capacity of $10^{8.95}$ CFUs mL^{-1} (95%CI = $10^{8.85}$ - $10^{9.05}$ CFUs mL^{-1}).

Relative invader growth rate predicts equilibrium proportion in the model community

Due to the variation in relative invader growth rate and equilibrium abundance across species combinations, we explored whether there was a link between relative invader growth rate, focal species, and equilibrium proportion. First, there was no significant interaction between the two predictors (ANOVA comparing models with and without an interaction between focal species and relative invader growth rate: χ^2_4= -0.62, P = 0.96). Instead, there was a positive relationship between the ability of a species to invade-from-rare and that species' long-term equilibrium proportion (Figure 3; ANOVA comparing models with and without relative invader growth rate as a predictor: χ^2_1= -10.298, P = 0.0013, R^2 = 0.69). Species with a higher relative invader growth rate had a higher equilibrium proportion in that community combination (Figure 3), and focal species did not explain a significant amount of the variation in equilibrium proportion (ANOVA comparing models with and without focal species as a predictor: χ^2_4= -1.70, P = 0.79).

Equilibrium proportion increases with relative invader growth rate in a simple model

To investigate whether this finding is likely to hold more generally, or is specific to our microbial community, we considered a simple two-species competitive community where interspecific competition is weaker than intraspecific competition (Figure 4). Our model recreated the experiments and metrics we used for our invasion-from-rare assays and long-term

306 coexistence experiments. Specifically, for each unique set of parameter values, we ran three
307 different simulations where the only difference was that both species either started with the
308 same abundance (Figure 4a), or species *1* (Figure 4b) or species *2* (Figure 4c) had 100-fold
309 lower starting density. With this single set of parameter values, it can be seen how equilibrium
310 proportion is unaffected by initial abundance, but short-term community dynamics change
311 substantially based on which species is invading from rare. Across a wide range of parameter
312 values, our simple model had a qualitatively equivalent pattern to the empirical data; a higher
313 relative invader growth rate resulted in a higher equilibrium proportion (Figure 4d). This proof-
314 of-principle approach indicates that this finding may apply to systems beyond our model
315 community.
316
317 *Species interactions across levels of diversity*
318 We used the data to explore the nature of species interactions between species pairs and also
319 how these changed at higher levels of diversity. Average pairwise interaction strength varied
320 between species (ANOVA of model with and without species as a predictor: $F_{4,19}$= 4.17, P =
321 0.018; Figure 5a). Most pairwise interactions were competitive (Figure 5a; Table S5), but
322 *Variovorax* sp. on average benefitted from the presence of other species (average abundance
323 relative to monoculture = 1.22, 95%CI = 1.01 - 1.43).
324 In combinations of three or four species, average indirect interactions differed
325 significantly between species (ANOVA of model with and without species as a predictor:
326 $F_{4,48}$=4.56, P = 0.0036; Table S6). An indirect interaction of the same sign would indicate that
327 interactions are stronger than would be expected from pairwise interactions calculated from
328 co-culture. In contrast, an indirect interaction of the opposite sign would indicate that
329 interactions are weaker than would be expected from pairwise interactions. *Achromobacter* sp.
330 had positive indirect interactions (*ii* = 0.2, 95%CI = 0.08 - 0.33), meaning that abundances of

331 *Achromobacter* sp. were higher in multi-species culture than those expected from the pairwise
332 interactions observed in co-culture. In contrast, *Ochrobactrum* sp. had negative indirect
333 interactions (*ii* = -0.13, 95%CI = -0.26 - -0.01), meaning that competitive interactions against
334 them intensify in multi-species culture. *Pseudomonas* sp., and *Stenotrophomonas* sp. had
335 indirect interaction estimates that had confidence intervals that spanned 0, and *Variovorax* sp.
336 had an average indirect interaction of just -0.01 even though it had the strong positive
337 interactions in pairwise culture. Despite the presence of some significant indirect interactions,
338 pairwise interactions were accurate predictors of species abundances (relative to monoculture)
339 at higher levels of diversity (>2 species; R^2 = 0.62; Figure 5b).
340

341 **Discussion**

342 Our 5 species model microbial community was extremely stable: 71/75 of our short-term
343 invasion assays, across community combinations of 2, 3, 4, and 5 species demonstrated
344 negative frequency dependence (i.e. the invader can re-establish from rare; Figure 1).
345 Moreover, we observed no extinctions when these combinations were cultured for 2, 3 or 4
346 weeks without replenishment, indicating longer term coexistence (Figure 2). Our results
347 showed that when species had a higher relative invasion growth rate, they reached a higher
348 equilibrium proportion in that community combination (Figure 3), and we found similar results
349 using a simple Lotka-Volterra competition model (Figure 4). This indicates that the value of
350 the invasion growth rate can predict equilibrium proportion. This relationship is especially
351 striking given the variation in strength and sign of both pairwise and indirect interactions we
352 observed between species.

353 Our work is, to our knowledge, the first experimental system to test the relationship
354 between relative invader growth rate and equilibrium proportion (Figure 3). Previous
355 theoretical work has predicted a positive link between them (Chisholm & Muller-Landau 2011)

because short-term growth rate correlates with competitive ability which then – at least partially - determines relative abundance. However, due to the difficulty of conducting fully factorial invasion-from-rare assays in a multi-species community, no work has experimentally tested this relationship (Grainger *et al.* 2019). Our work found a positive relationship between relative invader growth rate and equilibrium proportion (Figure 3) regardless of interaction sign and strength (Figure 5). The relationship is the same whether the species has on average negative (as in 4 of the 5 species in the community) or positive interactions (e.g. *Variovorax* sp.). Our modelling approach confirms our experimental results despite having exclusively competitive interactions (Figure 4). Consequently, measuring relative invader growth rate may be useful in predicting community properties in other experimental systems (Grainger *et al.* 2019). For example, we have shown that conducting full mutual invasibility tests of each species can predict long-term relative abundances of community members. This may be especially useful in - longer-living - experimental plant communities, where creating a stable community can take considerable time.

The relationship between short-term growth rate and long-term abundance can give insight into patterns in other ecological disciplines. In macroecology, it remains unknown whether growth rates predict equilibrium abundance across space (Holt 2019; Osorio-Olvera *et al.* 2019). There remains disagreement as to whether equilibrium abundances are higher in environments where growth rates are predicted to be higher - the niche centrality hypothesis (Martínez-Meyer *et al.* 2013; Dallas & Hastings 2018). Our empirical findings and simple model – where we assume no relationship between growth rates, carrying capacities, and species interactions – provide evidence that the density of populations should correlate with their growth rate in that environment.

Our study contributes to the growing body of work highlighting the prevalence of indirect interactions in diverse communities (Friedman *et al.* 2017; Mayfield & Stouffer 2017;

381 Sanchez-Gorostiaga *et al.* 2019). Like any single system, it is hard to generalise our findings
382 to natural ecosystems, this work provides some interesting insights. In our experiments, four
383 of the five species were negatively affected by another species in pairwise co-cultures, with
384 *Variovorax* sp. being the only species who on average benefitted from the presence of other
385 species (Figure 5d). We also observed significant indirect interactions in two of the five species
386 (*Achromobacter* sp. and *Ochrobactrum* sp.), where the presence of other species altered the
387 abundance of the focal species compared to that predicted solely from pairwise interactions.
388 Despite these significant indirect interactions, similar to previous studies (Friedman *et al.* 2017;
389 Mayfield & Stouffer 2017), our model from pairwise interactions alone can still explain
390 substantial variation (~60%) in focal species abundance across all community combinations.
391 The remaining unexplained variation is likely due, in part, to the indirect interactions (Mayfield
392 & Stouffer 2017) as well as temporal abundance variations that cannot be captured by snapshot
393 community sampling (Grainger *et al.* 2019; Pande *et al.* 2020).

394 Intriguingly, our model microbial community has no diversity-productivity
395 relationship, with total abundance in the 5 species combination being lower than some of the
396 monoculture abundances (Figure 2e). Specifically, *Stenotrophomonas* sp., the most abundant
397 species in monoculture, and *Ochrobactrum* sp. had competitive pairwise interactions which
398 became stronger in more diverse communities (negative indirect interactions). These
399 reinforcing indirect interactions likely contributed to the lack of a diversity-productivity
400 relationship in this system. This result is in contrast to most other studies using experimental
401 microbial communities which have reported positive diversity-productivity relationships
402 (Hodgson *et al.* 2002; Tilman *et al.* 2014; Goldford *et al.* 2018; Sanchez-Gorostiaga *et al.*
403 2019). Therefore, our study challenges the generality of these previous findings, especially as,
404 unlike the majority of other studies using synthetic microbial communities, we have
405 demonstrated that our community is stable.

The complex nature of our media (multiple carbon sources) makes it difficult to mechanistically understand how every single community combination remains stable. Relative invader growth values greater than one demonstrate negative frequency dependence (Figure 1). The mutual invasibility criterion by definition indicates stabilising mechanisms - such as niche differentiation between species - are promoting coexistence, with intraspecific interactions being more negative relative to interspecific interactions (Chesson 2000; Barabás *et al.* 2018). However, equalising mechanisms that minimise average fitness differences may also be contributing to the operation and strength of these stabilising mechanisms (Chesson 2000; Ellner *et al.* 2019; Grainger *et al.* 2019). For example, by using 1/64 TSB, we likely reduced fitness differences between the species, which prevented extinctions in all species combinations, from co-culture to the full 5 species combinations. Future work will attempt to disentangle stabilising and equalising mechanisms to better understand how this community coexists across levels of diversity.

Model microbial communities in the laboratory are increasingly used to test hypotheses in ecology and evolution (O'Malley *et al.* 2015; Goldford *et al.* 2018; Bell 2019), but it is rarely established whether the species coexist such that the community is stable. Here, we document this in a model 5 species community and confirm that its constituent species can coexist across all combinations of species and diversity. This model community provides an opportunity to explore how (co)evolution between community members impacts species interactions and community dynamics (Johnson & Stinchcombe 2007; Brockhurst & Koskella 2013). In exploring our model community, we empirically tested the link between relative invader growth rate and equilibrium proportion and demonstrated that a species' ability to re-establish from rare could predict its role in the community at steady state. This finding was confirmed using a simple model and highlights that findings from stable model microbial communities may be applicable to more complex, natural communities.

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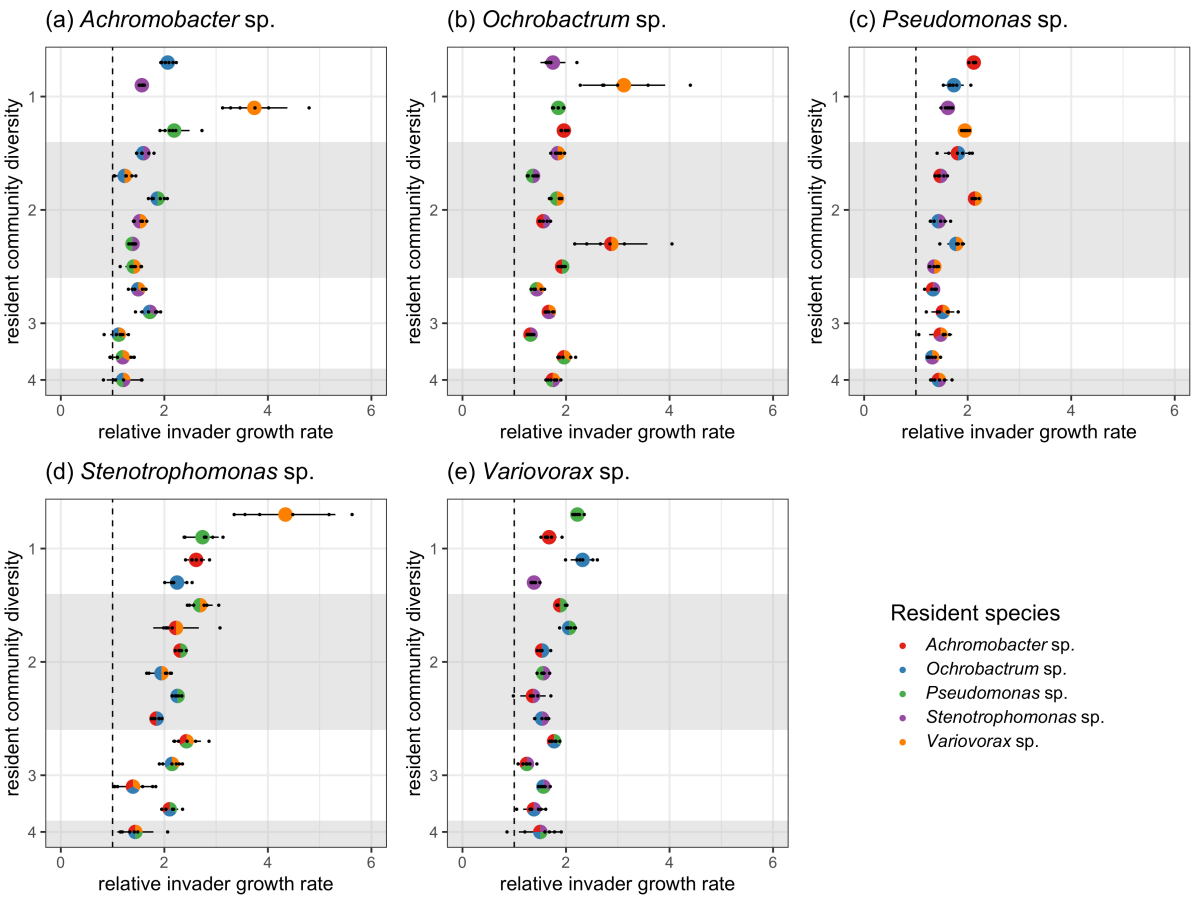
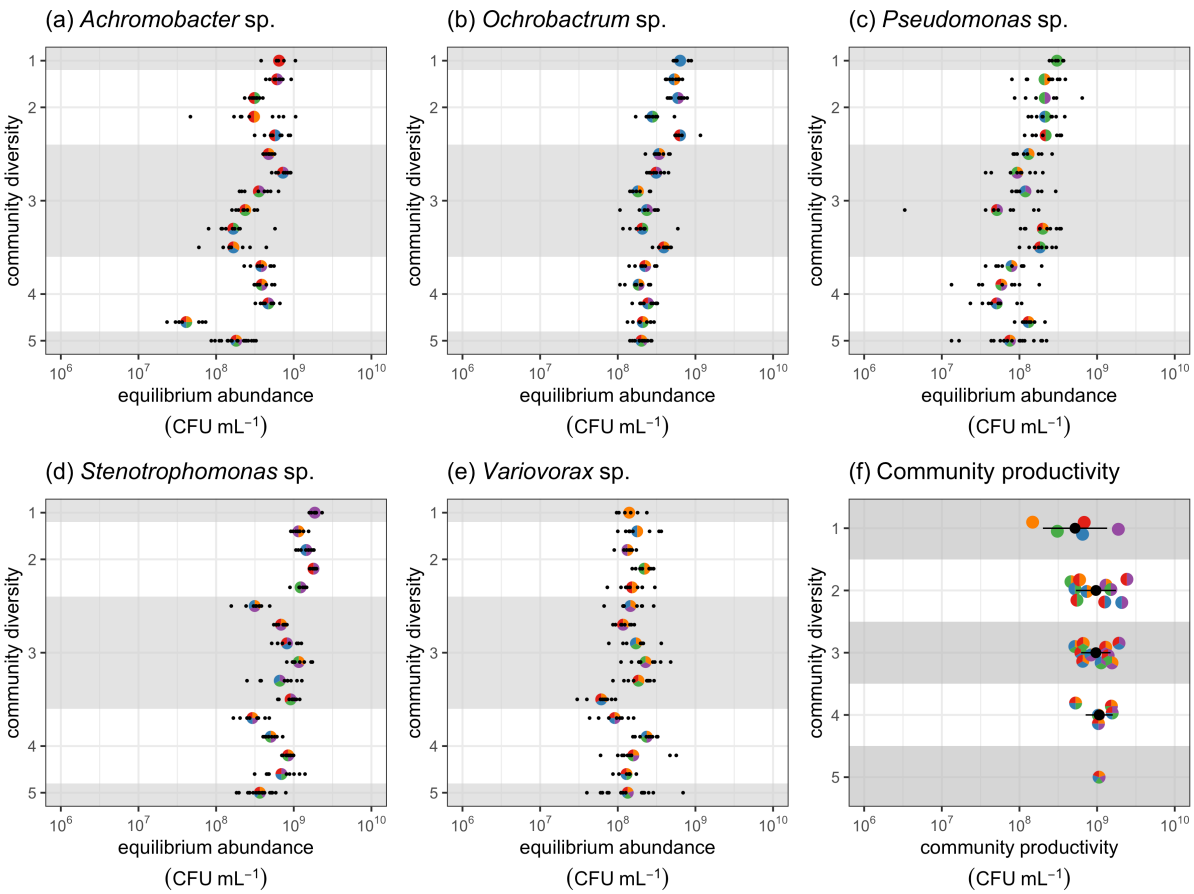


Figure 1. Effect of resident community diversity on relative invader growth rate of each species. Panels represent the invader species. Colours of pie points reflect the mix of the resident community. Black points are observed values of relative invader growth rate (n~6), pie points are the estimate of relative invader growth rate each one-sample t-test, and error bars represent lower and upper 95% confidence intervals. Shaded regions separate different levels of resident community diversity; dashed, vertical line drawn where relative invader growth rate = 1, values above which indicate the invading species can successfully re-establish from rare.



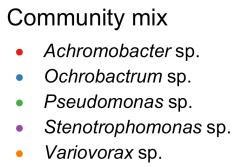


Figure 2. Effect of community combination and diversity on the equilibrium abundance of each species. Panels (a-e) represent the focal species; colours of pie points reflect the species in that community combination. Black points are the observed values of equilibrium abundance (n~9) while pie points are the mean of equilibrium abundance in that mix. Panel (f) demonstrates community productivity of each mix across diversity levels, where pie points are the mean community productivity for that mix, black points and error bars are the mean and standard deviation of productivity for each diversity level. Shaded regions separate different levels of community diversity.

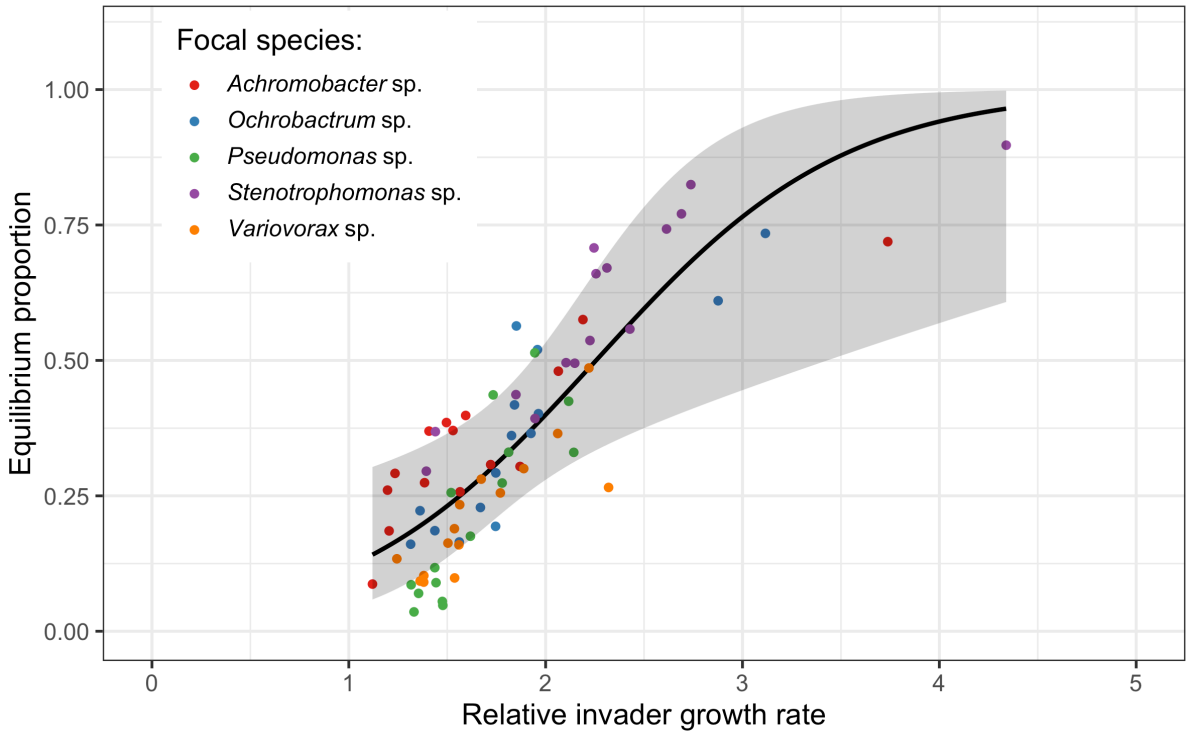


Figure 3. Relationship between relative invader growth rate and equilibrium proportion. There is a positive relationship between short-term relative invader growth rate and the equilibrium proportion a species reaches within the community. Points represent the mean value for each community combination (n=75). Lines and uncertainty bands represent the best fit line and 95% confidence intervals of the selected model respectively.

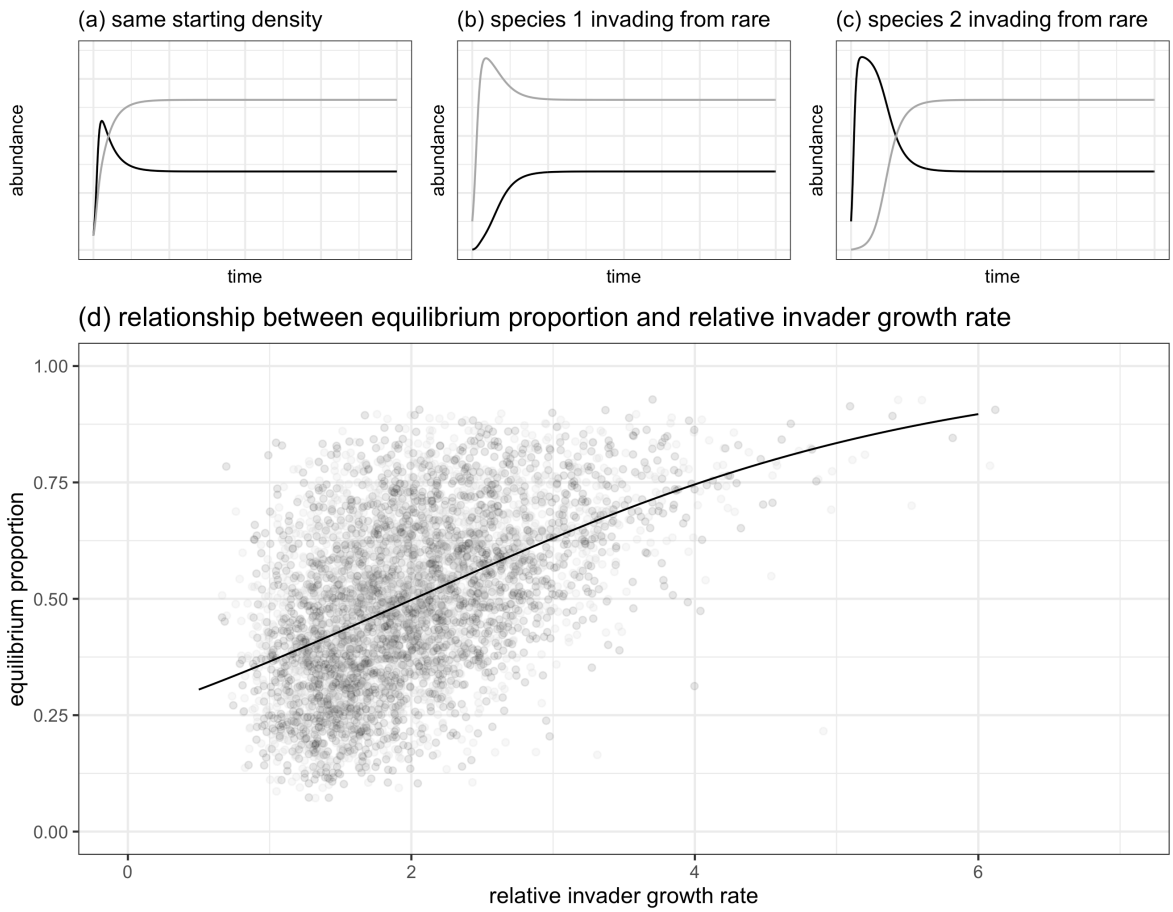


Figure 4. The relationship between relative invader growth rate and equilibrium proportion in Lotka-Volterra competition models. (a-c) Depiction of a run of the model with a single set of parameter values when (a) both species start at the same density, (b) species *1* invades from rare and (c) species *2* is invading from rare. Short-term abundance dynamics differ based on which species invades from rare, but equilibrium proportion does not. (d) Over 5000 simulated parameter values, there is a positive relationship between relative invader growth rate of the focal species and the equilibrium proportion it reaches during Lotka-Volterra competition. Points are estimates of species *1* or species *2* from independent simulations and the line is the best fit derived from a generalised linear model with a binomial error structure.

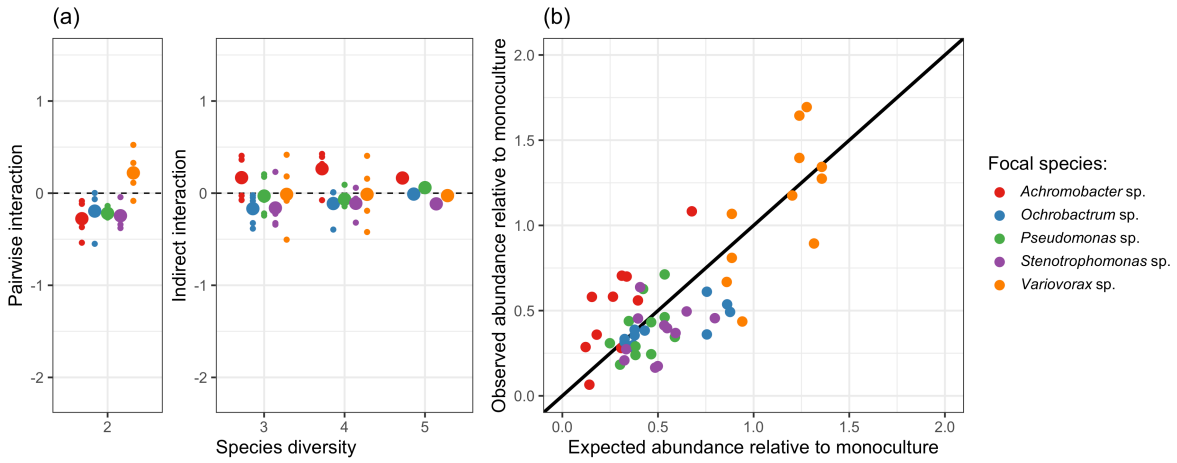


Figure 5. Variation in pairwise and indirect interactions in the model community. (a) Most pairwise species interactions are negative, apart from *Variovorax* sp. who on average benefits from the presence of other species. At higher levels of diversity, we identified substantial indirect interactions, but these did not change systematically across levels of diversity. (b) Pairwise interactions explained ~0.6 of the variation in the observed abundance relative to monoculture of the focal species across community combinations. Big points in (a) represent the mean of all individual combinations (small points). In (b) the line of best fit represents when pairwise interactions would perfectly predict abundance relative to monoculture at higher levels of diversity.

Supplementary Information: Relative invader growth rate predicts long-term equilibrium proportion in a stable, coexisting microbial community

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Table S1. The 46 bacterial strains used to establish our stable model community.

Code	Family	Genus
NMC6xB_4	Alcaligenaceae	Achromobacter
NMC5_19	Alcaligenaceae	Achromobacter
NMC1_18	Comamonadaceae	Acidovorax
NMC6_14	Microbacteriaceae	Agromyces
NMC2x_9	Micrococcaceae	Arthrobacter
NMC1_7	Micrococcaceae	Arthrobacter
NMC5_4	Bacillaceae	Bacillus
NMC5_14	Bacillaceae	Bacillus
NMC1B_24	Alcaligenaceae	Bordetella
NMC5xB_11	Alcaligenaceae	Bordetella
NMC5_15	Caulobacteraceae	Brevundimonas
NMC6x_4	Caulobacteraceae	Brevundimonas
NMC1B_19	Alcaligenaceae	Candidimonas
NMC6_15	Alcaligenaceae	Candidimonas
NMC2xB_19	Burkholderiaceae	Cupriavidus
NMC1_10	Burkholderiaceae	Cupriavidus
NMC1B_16	Hyphomicrobiaceae	Devosia
NMC5_2	Hyphomicrobiaceae	Devosia
NMC5x_2	Flavobacteriaceae	Flavobacterium
NMC5x_7	Flavobacteriaceae	Flavobacterium
NMC4_13	Planococcaceae	Lysinibacillus
NMC3_4	Planococcaceae	Lysinibacillus
NMC5B_21	Microbacteriaceae	Microbacterium
NMC5_10	Microbacteriaceae	Microbacterium
NMC4_4	Brucellaceae	Ochrobactrum
NMC6B_13	Cellulomonadaceae	Oerskovia
NMC2B_2	Cellulomonadaceae	Oerskovia
NMC4_22	Paenibacillaceae	Paenibacillus
NMC4x_9	Paenibacillaceae	Paenibacillus
NMC6xB_20	Rhodobacteraceae	Paracoccus
NMC5_5	Rhodobacteraceae	Paracoccus
NMC5_17	Sphingobacteriaceae	Pedobacter
NMC5_21	Alcaligenaceae	Pigmentiphaga
NMC5_3	Pseudomonadaceae	Pseudomonas
NMC6B_1	Pseudomonadaceae	Pseudomonas
NMC6B_4	Alcaligenaceae	Pusillimonas
NMC5_9	Alcaligenaceae	Pusillimonas
NMC4B_5	Rhizobiaceae	Rhizobium
NMC4B_1	Nocardiaceae	Rhodococcus
NMC4B_16	Nocardiaceae	Rhodococcus
NMC4B_3	Rhodocyclaceae	Shinella
NMC3xB_22	Staphylococcaceae	Staphylococcus
NMC5_6	Xanthomonodaceae	Stenotrophomonas
NMC5_23	Xanthomonodaceae	Stenotrophomonas
NMC1_21	Comamonadaceae	Variovorax
NMC6_9	Comamonadaceae	Variovorax

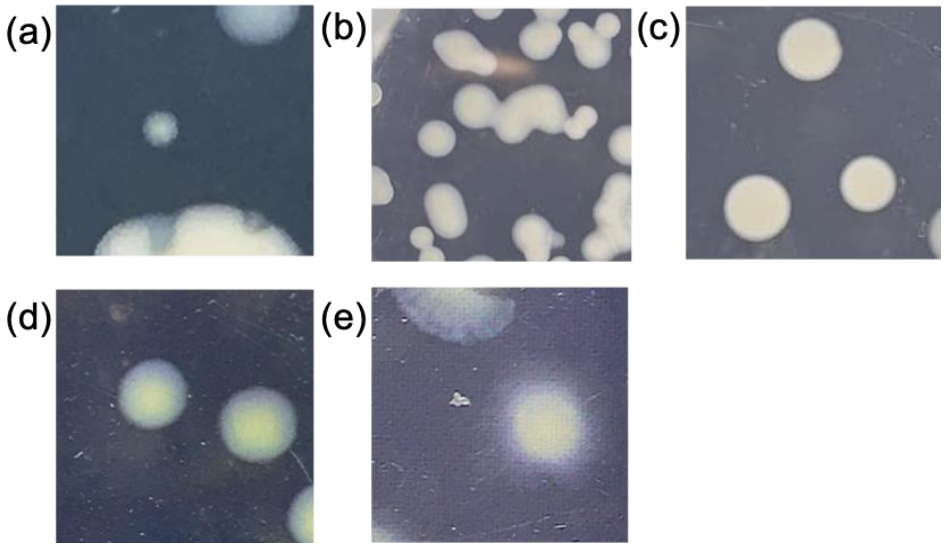

Figure S1. Unique colony morphologies of the five species. (a) *Achromobacter* sp., the smallest colony, circular and uniform in size. (b) *Ochrobactrum* sp. white/grey in colour and opaque. (c) *Pseudomonas* sp. is the largest colony, cream in colour and opaque. (d) *Stenotrophomonas* sp. is typically larger than *Achromobacter* sp. although variable in size and has a stronger colour. (e) *Variovorax* sp. has a spreading mat surrounding the colony and is variable in size.

Table S2. Equations from calibration curves to estimate each species' CFU uL^{-1} from optical density (OD) readings.

species	equation
Achromobacter sp.	(11676.OD - 291).16667
Ochrobactrum sp.	(5548.OD - 67).16667
Pseudomonas sp.	(546.OD - 20).16667
Stenotrophomonas sp.	(56625.OD - 2246).16667
Variovorax sp.	(955.OD - 30).16667

Table S3. Estimate of relative invader growth rate and 95% confidence intervals for each focal species in each community combination. Resident species are depicted by the first letter of their genus. Estimates and confidence intervals are calculated from a one-sample t-test.

invader species	resident species	resident diversity	estimated relative invader growth rate	95% CI lower	95% CI upper	*P*
Achromobacter sp.	P	1	2.19	1.89	2.49	<0.001
Achromobacter sp.	V	1	3.74	3.1	4.38	<0.001
Achromobacter sp.	S	1	1.56	1.52	1.61	<0.001
Achromobacter sp.	O	1	2.06	1.94	2.19	<0.001
Achromobacter sp.	P;V	2	1.41	1.25	1.57	0.001
Achromobacter sp.	P;S	2	1.38	1.34	1.43	<0.001
Achromobacter sp.	V;S	2	1.53	1.42	1.63	<0.001
Achromobacter sp.	O;P	2	1.87	1.72	2.02	<0.001
Achromobacter sp.	O;V	2	1.23	1.05	1.41	0.022
Achromobacter sp.	O;S	2	1.59	1.45	1.73	<0.001
Achromobacter sp.	P;V;S	3	1.2	0.96	1.43	0.084
Achromobacter sp.	O;P;V	3	1.12	0.95	1.29	0.124
Achromobacter sp.	O;P;S	3	1.72	1.52	1.92	<0.001
Achromobacter sp.	O;V;S	3	1.5	1.35	1.65	0.001
Achromobacter sp.	P;S;V;O	4	1.2	0.89	1.52	0.158
Ochrobactrum sp.	A	1	1.96	1.9	2.02	<0.001
Ochrobactrum sp.	P	1	1.85	1.75	1.95	<0.001
Ochrobactrum sp.	V	1	3.12	2.32	3.92	0.001
Ochrobactrum sp.	S	1	1.75	1.51	1.99	0.001
Ochrobactrum sp.	A;P	2	1.93	1.87	1.98	<0.001
Ochrobactrum sp.	A;V	2	2.88	2.18	3.57	0.001
Ochrobactrum sp.	A;S	2	1.56	1.47	1.66	<0.001
Ochrobactrum sp.	P;V	2	1.83	1.72	1.94	<0.001
Ochrobactrum sp.	P;S	2	1.36	1.27	1.45	<0.001
Ochrobactrum sp.	V;S	2	1.84	1.75	1.94	<0.001
Ochrobactrum sp.	A;P;V	3	1.96	1.81	2.11	<0.001
Ochrobactrum sp.	A;P;S	3	1.31	1.27	1.36	<0.001
Ochrobactrum sp.	A;V;S	3	1.67	1.6	1.74	<0.001
Ochrobactrum sp.	P;V;S	3	1.44	1.34	1.54	<0.001
Ochrobactrum sp.	P;S;A;V	4	1.75	1.63	1.86	<0.001
Pseudomonas sp.	V	1	1.94	1.88	2.01	<0.001
Pseudomonas sp.	S	1	1.62	1.53	1.7	<0.001
Pseudomonas sp.	O	1	1.73	1.54	1.92	<0.001
Pseudomonas sp.	A	1	2.12	2.07	2.17	<0.001
Pseudomonas sp.	V;S	2	1.35	1.28	1.43	<0.001
Pseudomonas sp.	O;V	2	1.78	1.61	1.95	<0.001
Pseudomonas sp.	O;S	2	1.44	1.27	1.61	0.001
Pseudomonas sp.	A;V	2	2.14	2.1	2.19	<0.001
Pseudomonas sp.	A;S	2	1.48	1.38	1.57	<0.001
Pseudomonas sp.	A;O	2	1.81	1.54	2.08	0.001
Pseudomonas sp.	O;V;S	3	1.32	1.22	1.42	0.001
Pseudomonas sp.	A;V;S	3	1.48	1.26	1.7	0.003
Pseudomonas sp.	A;O;V	3	1.52	1.3	1.74	0.002
Pseudomonas sp.	A;O;S	3	1.33	1.24	1.42	<0.001
Pseudomonas sp.	S;A;V;O	4	1.44	1.28	1.61	0.001
Stenotrophomonas sp.	O	1	2.25	2.04	2.45	<0.001
Stenotrophomonas sp.	A	1	2.61	2.44	2.78	<0.001
Stenotrophomonas sp.	P	1	2.74	2.43	3.05	<0.001
Stenotrophomonas sp.	V	1	4.34	3.37	5.3	<0.001
Stenotrophomonas sp.	A;O	2	1.85	1.77	1.93	<0.001
Stenotrophomonas sp.	P;O	2	2.26	2.18	2.33	<0.001
Stenotrophomonas sp.	V;O	2	1.95	1.73	2.17	<0.001
Stenotrophomonas sp.	P;A	2	2.31	2.24	2.38	<0.001
Stenotrophomonas sp.	V;A	2	2.23	1.78	2.67	0.001
Stenotrophomonas sp.	V;P	2	2.69	2.45	2.93	<0.001
Stenotrophomonas sp.	A;P;O	3	2.1	1.94	2.27	<0.001
Stenotrophomonas sp.	A;V;O	3	1.39	1	1.79	0.053
Stenotrophomonas sp.	P;V;O	3	2.15	1.96	2.34	<0.001
Stenotrophomonas sp.	P;V;A	3	2.43	2.15	2.71	<0.001
Stenotrophomonas sp.	P;A;V;O	4	1.44	1.09	1.79	0.024
Variovorax sp.	S	1	1.38	1.31	1.45	<0.001
Variovorax sp.	O	1	2.32	2.09	2.55	<0.001
Variovorax sp.	A	1	1.67	1.53	1.82	<0.001
Variovorax sp.	P	1	2.22	2.14	2.3	<0.001
Variovorax sp.	O;S	2	1.54	1.41	1.66	<0.001
Variovorax sp.	A;S	2	1.36	1.12	1.61	0.014
Variovorax sp.	P;S	2	1.56	1.48	1.64	<0.001
Variovorax sp.	A;O	2	1.54	1.45	1.63	<0.001
Variovorax sp.	P;O	2	2.06	1.94	2.18	<0.001
Variovorax sp.	P;A	2	1.89	1.8	1.98	<0.001
Variovorax sp.	A;O;S	3	1.38	1.17	1.59	0.006
Variovorax sp.	P;O;S	3	1.56	1.48	1.65	<0.001
Variovorax sp.	P;A;S	3	1.24	1.11	1.37	0.005
Variovorax sp.	A;P;O	3	1.77	1.7	1.84	<0.001
Variovorax sp.	P;S;A;O	4	1.5	1.09	1.92	0.028

Table S4. Estimates of mean abundance and 95% confidence intervals for each focal species across levels of diversity. Estimates and confidence intervals are calculated from the best model that included community diversity and focal species as independent predictors of species density. Degrees of freedom were calculated using the Kenward-Roger method.

species	diversity	df	mean abundance CFU mL^{-1}	95% CI lower	95% CI upper
Achromobacter sp.	1	77.32	$10^{8.77}$	$10^{8.57}$	$10^{8.97}$
Achromobacter sp.	2	70.92	$10^{8.67}$	$10^{8.55}$	$10^{8.79}$
Achromobacter sp.	3	70.88	$10^{8.46}$	$10^{8.35}$	$10^{8.57}$
Achromobacter sp.	4	71.11	$10^{8.36}$	$10^{8.24}$	$10^{8.48}$
Achromobacter sp.	5	65.03	$10^{8.29}$	$10^{8.1}$	$10^{8.48}$
Ochrobactrum sp.	1	76.92	$10^{8.76}$	$10^{8.56}$	$10^{8.95}$
Ochrobactrum sp.	2	70.88	$10^{8.66}$	$10^{8.54}$	$10^{8.78}$
Ochrobactrum sp.	3	70.92	$10^{8.45}$	$10^{8.34}$	$10^{8.56}$
Ochrobactrum sp.	4	71.07	$10^{8.35}$	$10^{8.23}$	$10^{8.47}$
Ochrobactrum sp.	5	65.01	$10^{8.28}$	$10^{8.09}$	$10^{8.47}$
Pseudomonas sp.	1	76.94	$10^{8.37}$	$10^{8.17}$	$10^{8.57}$
Pseudomonas sp.	2	71.36	$10^{8.27}$	$10^{8.15}$	$10^{8.39}$
Pseudomonas sp.	3	70.92	$10^{8.06}$	$10^{7.95}$	$10^{8.18}$
Pseudomonas sp.	4	71.03	$10^{7.96}$	$10^{7.84}$	$10^{8.08}$
Pseudomonas sp.	5	65.04	$10^{7.9}$	$10^{7.71}$	$10^{8.09}$
Stenotrophomonas sp.	1	76.96	$10^{9.17}$	$10^{8.98}$	$10^{9.37}$
Stenotrophomonas sp.	2	71.51	$10^{9.07}$	$10^{8.95}$	$10^{9.2}$
Stenotrophomonas sp.	3	70.97	$10^{8.87}$	$10^{8.75}$	$10^{8.98}$
Stenotrophomonas sp.	4	71.07	$10^{8.76}$	$10^{8.64}$	$10^{8.89}$
Stenotrophomonas sp.	5	65.05	$10^{8.7}$	$10^{8.51}$	$10^{8.89}$
Variovorax sp.	1	76.92	$10^{8.44}$	$10^{8.25}$	$10^{8.64}$
Variovorax sp.	2	70.99	$10^{8.34}$	$10^{8.22}$	$10^{8.46}$
Variovorax sp.	3	70.83	$10^{8.14}$	$10^{8.02}$	$10^{8.25}$
Variovorax sp.	4	71.07	$10^{8.03}$	$10^{7.91}$	$10^{8.16}$
Variovorax sp.	5	65.01	$10^{7.97}$	$10^{7.78}$	$10^{8.16}$

Table S5. Estimates and 95% confidence intervals of average pairwise interaction for each species.

species	df	average pairwise interaction	95% CI lower	95% CI upper
Achromobacter sp.	15	0.72	0.51	0.94
Ochrobactrum sp.	15	0.81	0.59	1.02
Pseudomonas sp.	15	0.78	0.57	1
Stenotrophomonas sp.	15	0.76	0.54	0.97
Variovorax sp.	15	1.22	1.01	1.43

Table S6. Estimates and 95% confidence intervals of average indirect interaction for each species.

species	df	average indirect interaction	95% CI lower	95% CI upper
Achromobacter sp.	50	0.2	0.08	0.33
Ochrobactrum sp.	50	-0.13	-0.26	-0.01
Pseudomonas sp.	50	-0.04	-0.16	0.09
Stenotrophomonas sp.	50	-0.14	-0.26	-0.02
Variovorax sp.	50	-0.01	-0.14	0.11