

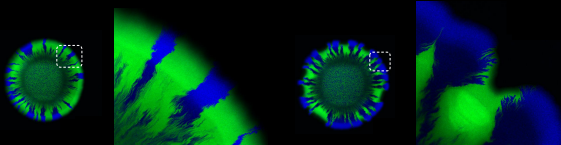
# Hyphae promote the maintenance of diversity/intermixing

[Click here to view original image](#)

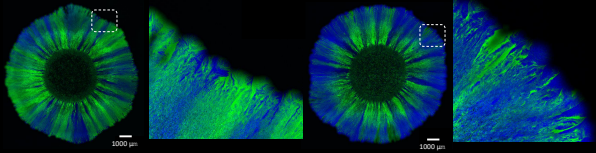
Competition

Cross-feeding

Without hyphae

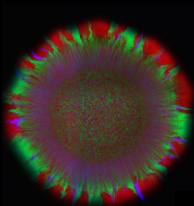


With hyphae

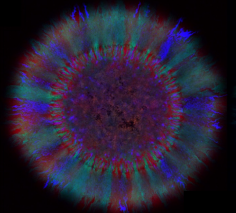


# Hyphae promote the emergence of functional novelty

Without hyphae



With hyphae



---

1 **COVER PAGE**

2

3 **Title**

4 Fungal hyphae regulate bacterial diversity and plasmid-mediated functional novelty during  
5 range expansion

6

7 **Authors**

8 Chujin Ruan<sup>1,2,7</sup>, Josep Ramoneda<sup>2,3,7</sup>, Guram Gogia<sup>4,2</sup>, Gang Wang<sup>1,5</sup>, David R. Johnson<sup>2,6,\*</sup>

9

10 **Affiliations**

11 <sup>1</sup>College of Land Science and Technology, China Agricultural University, 100193, Beijing,  
12 China; <sup>2</sup>Department of Environmental Microbiology, Swiss Federal Institute of Aquatic  
13 Science and Technology (Eawag), CH-8600 Dübendorf, Switzerland; <sup>3</sup>Cooperative Institute  
14 for Research in Environmental Sciences, University of Colorado, Boulder, CO 80309, USA;  
15 <sup>4</sup>Department of Environmental Systems Science, Swiss Federal Institute of Technology, CH-  
16 8092 Zürich, Switzerland; <sup>5</sup>National Black Soil & Agriculture Research, China Agricultural  
17 University, 100193, Beijing, China; <sup>6</sup>Institute of Ecology and Evolution, University of Bern,  
18 3012 Bern, Switzerland. <sup>7</sup>These authors contributed equally.

19

20 **\*Lead Contact**

21 David R. Johnson, [david.johnson@eawag.ch](mailto:david.johnson@eawag.ch)

---

## 22 **SUMMARY**

23 The amount of bacterial diversity present on many surfaces is enormous, yet how these  
24 levels of diversity persist in the face of the purifying processes that occur as bacterial  
25 communities expand across space (referred to here as range expansion) remains enigmatic.  
26 We shed light on this apparent paradox by providing mechanistic evidence for a strong role  
27 of fungal hyphae-mediated dispersal on regulating bacterial diversity during range  
28 expansion. Using pairs of fluorescently labelled bacterial strains and a hyphae-forming  
29 fungal strain that expand together across a nutrient-amended surface, we show that a  
30 hyphal network increases the spatial intermixing and extent of range expansion of the  
31 bacterial strains. This is true regardless of the type of interaction (competition or resource  
32 cross-feeding) imposed between the bacterial strains. We further show that the underlying  
33 cause is that flagellar motility drives bacterial dispersal along the hyphal network, which  
34 counteracts the purifying effects of ecological drift at the expansion frontier. We finally  
35 demonstrate that hyphae-mediated spatial intermixing increases the conjugation-mediated  
36 spread of plasmid-encoded antibiotic resistance. In conclusion, fungal hyphae are important  
37 regulators of bacterial diversity and promote plasmid-mediated functional novelty during  
38 range expansion in an interaction-independent manner.

39

## 40 **Keywords**

41 Range expansion; microbial dispersal; biofilms; fungal hyphae; bacterial diversity; bacterial  
42 motility; plasmid conjugation; horizontal gene transfer; antibiotic resistance

---

## 43 INTRODUCTION

44

45 Surface-associated bacterial communities are ubiquitous across our planet and have  
46 important roles in biogeochemical cycles, ecosystem processes, agriculture, environmental  
47 sustainability, and human health and disease<sup>1-3</sup>. A universal feature of all communities is  
48 that they must, at some point in their existence, undergo range expansion<sup>4,5</sup>. Range  
49 expansion refers to the spreading of organisms across space as a consequence of their  
50 reproduction and dispersal. During the range expansion process, communities undergo  
51 irreversible diversity loss due to small effective population sizes and strong ecological drift  
52 at the expansion frontier, where only a few individuals positioned at the frontier contribute  
53 to further range expansion<sup>4-9</sup>.

54

55 The universality of range expansion and its associated negative effects on the maintenance  
56 of diversity raises an important paradox. Many surface-associated bacterial communities are  
57 incredibly diverse, where soil and host-associated microbiomes may contain many hundreds  
58 to thousands of bacterial taxa<sup>10-12</sup>. How are these levels of bacterial diversity maintained in  
59 the face of the purifying effects that occur during range expansion? Resource cross-feeding  
60 between cell-types is one process that can counteract these effects, where cross-feeding  
61 tends to maintain higher levels of spatial intermixing of different cell-types, and thus higher  
62 levels of diversity<sup>13-15</sup>. However, cross-feeding does not universally occur between all  
63 bacterial cell-types; rather, competition is also pervasive<sup>16-18</sup>. Are there mechanisms that  
64 counteract the purifying effects of ecological drift at the expansion frontier that are  
65 independent of interactions? Resource supply<sup>19</sup>, metabolite toxicity<sup>20</sup>, initial cell densities<sup>21</sup>,  
66 initial spatial configurations of cells<sup>22,23</sup>, and spatial structure<sup>24</sup> can all affect short-term  
67 spatial intermixing during range expansion in an interaction-independent manner, but they



---

68 either have no effects on long-term spatial intermixing or have effects that are too small to  
69 account for the levels of diversity observed in nature. In spatially heterogenous  
70 environments such as soils or the gut lumen, spatial isolation maintains diversity by  
71 preventing competitive exclusion of populations<sup>25</sup>. However, at the scale of each isolated  
72 population, the effects of drift during proliferation are still expected to negatively impact  
73 diversity. Clearly, further knowledge on the processes that maintain bacterial diversity  
74 during range expansion is needed to resolve this paradox.

75

76 We hypothesized here that bacterial dispersal via fungal hyphae can counteract the  
77 purifying effects of ecological drift at the expansion frontier during range expansion in an  
78 interaction-independent manner. Bacteria and fungi co-occur in a myriad of environments,  
79 including soils<sup>26</sup>, host-associated microbiomes<sup>27-30</sup>, and a variety of biotechnological  
80 applications<sup>31</sup>. Importantly, fungal hyphae can promote bacterial dispersal on virtually any  
81 surface where they co-occur, where the water films surrounding fungal hyphae provide  
82 hydrated environments that enable active bacterial motility<sup>32-37</sup>. This, in turn, has important  
83 consequences for the functioning of surface-associated bacterial communities, where  
84 increased bacterial dispersal can improve access to growth resources<sup>38-40</sup>, promote  
85 conjugation-mediated plasmid transfer<sup>41</sup>, enable escape from predators<sup>42</sup>, and promote  
86 transport of phages<sup>43</sup>. While not established for fungal hyphae, dispersal and its trade-offs  
87 with growth rate can promote the maintenance of diversity by reducing interspecific  
88 competition<sup>44-49</sup>. Together, this evidence suggests that increased bacterial dispersal along  
89 fungal hyphae could help resolve the paradox between observed levels of surface-  
90 associated bacterial diversity and the universal processes that drive diversity loss during  
91 range expansion.

92

---

93 To test our hypothesis, we performed range expansion experiments with pairs of bacterial  
94 strains in the presence or absence of a hyphae-forming fungus. We expected that the thin  
95 water- film networks surrounding fungal hyphae would serve as dispersal pathways that  
96 allow bacteria to escape the effects of ecological drift at the expansion frontier and occupy  
97 uncolonized space, leading to higher spatial intermixing. Higher spatial intermixing indicates  
98 higher bacterial diversity, as more individuals are able to emigrate from the founder  
99 population and contribute to active range expansion<sup>7,15,20</sup>. We next tested whether the  
100 effects of fungal hyphae on spatial intermixing are independent of the type of interaction  
101 imposed between the bacterial strains (competition or resource cross-feeding). We then  
102 tested defects in pili- and flagella-mediated motility to identify the active dispersal  
103 mechanism along fungal hyphae. Finally, we addressed the consequences of hyphae-  
104 mediated spatial intermixing on the spread of plasmid-encoded functional novelty, with  
105 specific focus on the spread of antibiotic resistance.

106

## 107 **RESULTS**

108

109 *Fungal hyphae counteract the loss of spatial intermixing of competing bacterial strains*  
110 *during range expansion.*

111

112 We first tested whether the presence of fungal hyphae can counteract the loss of spatial  
113 intermixing of competing bacterial strains during range expansion, and thus counteract the  
114 loss of bacterial diversity. To test this, we used two pairs of competing bacterial strains  
115 (*Pseudomonas aeruginosa* PAO1-*gfp* and PAO1-*rfp*, and *Pseudomonas stutzeri* A1601-*egfp*  
116 and A1601-*ecfp*), where each strain expresses a different fluorescent protein but is  
117 otherwise genetically and phenotypically identical to its paired strain (Figure 1A and Table

---

118 S1). Both strains have the complete denitrification pathway and will compete for nitrate  
119 ( $\text{NO}_3^-$ ) when grown together under anoxic conditions with an exogenous supply of nitrate.  
120 We conducted experiments where we mixed the pairs either with or without the hyphae-  
121 forming fungus *Penicillium* sp. laika and inoculated them onto agar plates amended with  
122 nitrate (Figure 1B). We next incubated the agar plates for two days under oxic conditions  
123 (Figure 1B), which allowed the fungus to form a dense hyphal network (Figure S1A) and the  
124 bacterial strains to begin growing and dispersing across the hyphal network (Figure S1B). We  
125 finally transferred the agar plates to anoxic conditions to induce nitrate competition  
126 between the bacterial strains and incubated them for an additional two days (Figure 1B).  
127 The fungus could not grow under anoxic conditions (Figure S1C) while the bacterial strains  
128 could continue growing with nitrate and disperse across the hyphal network.

129

130 Consistent with our expectation, we found that the presence of fungal hyphae can indeed  
131 counteract the loss of spatial intermixing between competing bacterial strains at the  
132 expansion frontier (Figure 2). Here and throughout, we defined the expansion frontier as a  
133 35  $\mu\text{m}$ -wide band located at the leading edge of the expansion area, which reflects growth  
134 during the anoxic phase and was selected based on experimental measures of the width of  
135 the actively growing layer of bacterial cells in similar experimental setups<sup>19</sup>. For the *P.*  
136 *aeruginosa* PAO1 strains, the presence of fungal hyphae significantly increased spatial  
137 intermixing at the expansion frontier (two-sample two-sided Welch tests;  $P < 0.03$ ,  $n = 5$ )  
138 (Figures 2E [local scale Fourier transform method] and 2G [intersection method]), an effect  
139 that progressively weakened at increasing distances behind the expansion frontier (Figures  
140 S2A [local scale Fourier transform method] and S3A [intersection method]). The increase in  
141 intermixing by fungal hyphae was further amplified when we quantified intermixing at  
142 intermediate scales (Figure S4), but we focused here on the local scale as this scale operates

---

143 closest to the finest scales of intermixing that we observed experimentally. For the *P.*  
144 *stutzeri* A1601 strains, fungal hyphae also significantly increased spatial intermixing at the  
145 expansion frontier (two-sample two-sided Welch tests;  $P < 0.004$ ,  $n = 5$ ) (Figures 2F [local  
146 scale Fourier transform method] and 2H [intersection method]), and this effect persisted  
147 across the entire expansion area (Figures S2B [local scale Fourier transform method] and  
148 S3B [intersection method]).

149

150 We further found that the presence of fungal hyphae increased consortium-level expansion  
151 distances of competing bacterial strains (Figures S5A and S5B). The expansion radii were  
152 significantly greater in the presence of fungal hyphae than in the absence for both pairs of  
153 bacterial strains (two-sample two-sided Welch tests;  $P = 0.02$  for the *P. aeruginosa* PAO1  
154 pair,  $P = 1 \times 10^{-8}$  for the *P. stutzeri* A1601 pair,  $n = 5$ ) (Figures S5A and S5B). This could be  
155 caused either by improved dispersal or improved growth of the bacterial strains when in the  
156 presence of fungal hyphae. To discriminate between these two possibilities, we quantified  
157 the total biomass of the pairs of competing bacterial strains when in the presence or  
158 absence of fungal hyphae at the end of the range expansion experiment. For the *P.*  
159 *aeruginosa* PAO1 strains, the total biomass was statistically identical when in the presence  
160 or absence of fungal hyphae (two-sample two-sided Welch test;  $P = 0.07$ ,  $n = 5$ ) (Figure S5D).  
161 For the *P. stutzeri* A1601 strains, the total biomass was significantly lower when in the  
162 presence of fungal hyphae (two-sample two-sided Welch test;  $P = 0.0001$ ,  $n = 5$ ) (Figure  
163 S5E). Thus, the increased extent of range expansion when in the presence of fungal hyphae  
164 was not caused by improved growth of the bacterial strains (e.g., via positive metabolic  
165 interactions with the fungus), but was instead likely caused by improved dispersal across the  
166 hyphal network.

167

---

168 *Fungal hyphae counteract the loss of spatial intermixing during range expansion in an*  
169 *interaction-independent manner.*

170

171 We next tested whether the effects of fungal hyphae on bacterial diversity during range  
172 expansion extend beyond competitive interactions. To test this, we used isogenic mutant  
173 strains of *P. stutzeri* A1601 (strains A1602 and A1603) that cross-feed the metabolic  
174 intermediate nitrite (NO<sub>2</sub><sup>-</sup>) when grown under anoxic conditions with nitrate (NO<sub>3</sub><sup>-</sup>) as the  
175 growth-limiting resource (Figures 1A and 3)<sup>50</sup>. As we observed for the competing pairs of  
176 bacterial strains, the fungal hyphae significantly increased spatial intermixing at the  
177 expansion frontier for the cross-feeding pair (two-sample two-sided Welch test;  $P < 7 \times 10^{-6}$ ,  
178  $n = 5$ ) (Figures 3C [local-scale Fourier transform method] and 3D [intersection method]). This  
179 effect size is remarkably consistent with that measured for the competing pair of *P. stutzeri*  
180 A1601 strains (Figures 2F and 3C), indicating that the effect size is largely interaction-  
181 independent. Also consistent with the competing pairs of bacterial strains, we found that  
182 fungal hyphae increased consortium-level expansion distances (two-sample two-sided  
183 Welch test;  $P = 1 \times 10^{-9}$ ,  $n = 5$ ) (Figure S5C) while reducing total biomass (two-sample two-  
184 sided Welch test;  $P = 0.003$ ,  $n = 5$ ) (Figure S5F). Thus, the presence of fungal hyphae had  
185 positive effects on bacterial diversity and the extent of consortium-level expansion in an  
186 interaction-independent manner.

187

188 *Flagellum-mediated motility is essential for improved bacterial dispersal in the presence of*  
189 *fungal hyphae.*

190

191 To identify the mechanism of bacterial dispersal along fungal hyphae, we conducted  
192 additional range expansion experiments with *P. aeruginosa* PAO1 strains that have a loss-of-

---

193 function deletion in either the type IV pilus-encoding *pilA* gene (strain PAO1- $\Delta$ *pilA*-*rfp*) or  
194 the flagellum-encoding *fliC* gene (strain PAO1- $\Delta$ *fliC*-*rfp*). We found that the ancestral PAO1-  
195 *rfp* and the PAO1- $\Delta$ *pilA*-*rfp* strains dispersed along the hyphal network whereas the PAO1-  
196  $\Delta$ *fliC*-*rfp* strain did not (Figures 4A-4C). The radii of the range expansions formed by the  
197 PAO1- $\Delta$ *fliC*-*rfp* strain were significantly smaller than those formed by the PAO1- $\Delta$ *pilA*-*rfp*  
198 strain (two-sample two-sided Welch test;  $P = 3 \times 10^{-6}$ ,  $n = 3$ ) and the ancestral PAO1-*rfp*  
199 strain (two-sample two-sided Welch test;  $P = 4 \times 10^{-7}$ ,  $n = 3$ ) (Figure 4D). The radii of the  
200 range expansions formed by the PAO1- $\Delta$ *pilA*-*rfp* strain were also significantly smaller than  
201 those formed by the ancestral PAO1-*rfp* strain (Welch two-sample two-sided t-test;  $P =$   
202  $0.003$ ,  $n = 3$ ), albeit with a smaller effect size (Figure 4D). In contrast, the radii of the range  
203 expansions were statistically identical for all the strains in the absence of *Penicillium* sp.  
204 *laika* (Welch two-sample two-sided t-test;  $P > 0.5$ ,  $n = 3$ ) (Figure 4D). We performed  
205 additional experiments to rule out biological interactions with *Penicillium* sp. *laika*. Briefly,  
206 we allowed the strains to come into contact with a 5  $\mu$ m-diameter glass fiber during range  
207 expansion, where the glass fiber promotes the formation of a thin aqueous water film.  
208 Consistent with our experiments with fungal hyphae, the ancestral PAO1-*rfp* and the PAO1-  
209  $\Delta$ *pilA*-*rfp* strains dispersed along the glass fiber while the PAO1- $\Delta$ *fliC*-*rfp* strain did not  
210 (Figures 4E-4G). Moreover, when using pairs of competing or cross-feeding bacterial strains,  
211 we found that the strains co-migrate along the glass fiber (Figure 5). Thus, a functional  
212 flagellum is essential to explain the improved dispersal across fungal hyphae, and the  
213 improved dispersal and intermixing are likely consequences of the hydrodynamic  
214 environment created by fungal hyphae rather than a consequence of biological interactions  
215 with the fungus itself.  
216

---

217 *Topographical effects cannot explain the effects of fungal hyphae on the maintenance of*  
218 *diversity.*

219

220 In addition to increasing the dispersal and expansion range of bacterial individuals, the  
221 complex topography of the hyphal network could also have positive effects on the  
222 maintenance of diversity via increased spatial heterogeneity<sup>51,52</sup>. Spaces between the  
223 hyphae could spatially segregate distinct bacterial populations and allow their simultaneous  
224 occurrence. We tested for this effect by quantifying the degree of spatial intermixing  
225 between pairs of *P. aeruginosa* PAO1 strains that are unable to produce a functional  
226 flagellum (strains PAO1- $\Delta$ fliC-rfp and PAO1- $\Delta$ fliC-gfp). In the absence of a functional  
227 flagellum, the spatial intermixing of such populations should be solely due to the hyphal  
228 network topography and ecological drift. We found that spatial intermixing in the presence  
229 or absence of a hyphal network is statistically identical (two-sample two-sided Welch test;  $P$   
230 = 0.4,  $n = 3$ ) (Figures 6A-6C), indicating a lack of apparent topographical effects. Our  
231 scanning electron microscopy images support this finding by showing many instances where  
232 bacterial cells migrated over the hyphae and dispersed across the spaces between hyphae  
233 (Figure 6D). Thus, topography cannot explain our experimentally observed effects of fungal  
234 hyphae on the maintenance of diversity during bacterial range expansion.

235

236 *Fungal hyphae promote conjugation-mediated functional novelty.*

237

238 We finally tested whether the presence of fungal hyphae can promote plasmid conjugation  
239 between strains, and thus promote the emergence of functional novelty. Our reasoning is  
240 that fungal hyphae increase the spatial intermixing of bacterial strains (Figures 2 and 3),  
241 which in turn increases the number of interspecific cell-cell contacts and the extent of

---

242 plasmid conjugation. To test this, we used the competing pair of *P. stutzeri* A1601 strains  
243 where one expresses red (*P. stutzeri* A1601-*ech*) and the other expresses green (*P. stutzeri*  
244 A1601-*egfp*) fluorescent protein (Table S1). Both of these fluorescent proteins are encoded  
245 by genes introduced into the same neutral site in the chromosome<sup>15</sup>. Both strains also have  
246 a loss-of-function mutation in the competence-enabling *comA* gene (Table S1), which  
247 prevents transformation of these fluorescent protein-encoding genes between the bacterial  
248 strains<sup>50</sup>. We then introduced plasmid pAR145 into *P. stutzeri* A1601-*egfp*, which encodes  
249 for chloramphenicol resistance and cyan fluorescent protein (Table S1), and performed  
250 range expansions in the absence of chloramphenicol (*i.e.*, we did not impose selection for  
251 transconjugants). Areas within the expansion region where both red and cyan (but not  
252 green) fluorescent proteins are expressed indicate regions where pAR145 successfully  
253 conjugated into *P. stutzeri* A1601-*ech*.

254

255 Using the same experimental design as for our other range expansion experiments, we  
256 observed conjugation of pAR145 to *P. stutzeri* A1601-*ech* both in the absence (Figures 7A  
257 and 7B) and presence (Figures 7C and 7D) of fungal hyphae. These events are identifiable as  
258 blue patches within the expansion area. As expected, fungal hyphae increased the extent of  
259 plasmid conjugation both within the entire area (two-sample two-sided Welch-test;  $P = 4 \times$   
260  $10^{-5}$ ,  $n = 5$ ) (Figure 7E) and within only the expansion area (two-sample two-sided Welch-  
261 test;  $P = 0.007$ ,  $n = 5$ ) (Figure 7F). Thus, the presence of fungal hyphae not only counteracts  
262 the loss of diversity during bacterial range expansion, but also promotes the emergence of  
263 plasmid-mediated functional novelty.

264

## 265 **DISCUSSION**

266



---

267 Our study revealed that hyphal networks can regulate bacterial diversity during range  
268 expansion. Ecological drift at the expansion frontier and resource limitations behind the  
269 expansion frontier have strong purging effects on the diversity of surface-associated  
270 microbial communities<sup>7,8,19,53</sup>, which is apparent in the highly segregated spatial patterns  
271 that characterize competition-dominated systems<sup>9</sup>. The hyphal network increases spatial  
272 intermixing by increasing the dispersal capabilities of individuals that would otherwise only  
273 disperse via cell-shoving and short-range twitching. These factors promote the simultaneous  
274 proliferation of larger numbers of spatially segregated bacterial populations, reflecting the  
275 maintenance of diversity during range expansion (Figures 2 and 3). Our results therefore  
276 provide evidence that frequent long-distance dispersal can increase expansion speeds and  
277 promote the maintenance of microbial diversity over time<sup>49,54-56</sup>.

278

279 In our system, bacterial dispersal along fungal hyphae is driven by active flagellar motility  
280 that enables individuals to colonize unoccupied space (Figures 4 and 5), which accelerates  
281 range expansion and alleviates the effects of ecological drift. We found that the mechanism  
282 mediating this process is the micro-hydrophysical environment created by fungal hyphae,  
283 which we demonstrated using a glass fiber as a simple physical surrogate of fungal hyphal  
284 structure that allowed us to exclude specific biological interactions and processes (e.g.,  
285 chemotaxis<sup>57</sup> or secretion of signal inducing molecules<sup>58</sup>) (Figures 4 and 5). Under the  
286 physical conditions created by the glass fiber, we observed co-migration and increased  
287 intermixing of bacterial strains regardless of the interactions that occurred between them  
288 (Figure 5). This means that as long as fungal hyphae and their associated thin water films are  
289 present (which may vary with hydration conditions), local bacterial diversity and intermixing  
290 can be maintained regardless of whether the fungus is physiologically active or not.

291

---

292 In a system where the probability of dispersal upon contact with a fungal hypha is low and  
293 the available nutrients are not sufficient to support rapid proliferation, the presence of  
294 fungal hyphae will also create a heterogeneous topography that could spatially isolate  
295 different populations and increase microbial diversity globally<sup>59</sup>. For example, recent  
296 research demonstrated that differential dispersal across hyphal networks by bacteria with  
297 different motility strategies determined the diversity and composition of cheese rind  
298 microbiomes<sup>36</sup>. Besides trait differences between taxa, the maintenance of bacterial  
299 diversity across surfaces with heterogeneous topographies such as soils or activated sludge  
300 can be controlled by host-mediated dispersal (e.g. invertebrates)<sup>60,61</sup>, transient changes to  
301 hydration conditions<sup>62</sup>, or by the pore structure of the matrix where range expansion  
302 occurs<sup>25</sup>. These factors can rescue microbial populations undergoing extinction due to  
303 ecological drift by promoting spatial isolation. Our study demonstrates that hyphal networks  
304 and their associated thin water films alone can promote the maintenance of microbial  
305 diversity during range expansion without the need for such topographical effects (Figure 6).  
306 Future work could improve the transfer of our findings to natural systems by adding  
307 additional processes to the experimental system such as periodic evaporation/hydration or  
308 the addition of burrowing eukaryotes, and then quantify the strength of the fungal hyphae-  
309 mediated effects on counteracting drift in the presence or absence of these additional  
310 processes.

311

312 We finally found that bacterial communities that expand in the presence of hyphal networks  
313 have an increased extent of plasmid conjugation between local populations during range  
314 expansion (Figure 7). A previous study demonstrated that fungal hyphae enable the long-  
315 range movement of plasmid donors and potential recipients that are otherwise spatially  
316 isolated in separate colonies<sup>41</sup>. This increases the number of interspecific cell-cell contacts

---

317 and promotes plasmid conjugation<sup>41</sup>. Hydration dynamics in unsaturated environments can  
318 also enable the long-range movement of plasmid donors and potential recipients, increase  
319 the number of interspecific cell-cell contacts, and promote plasmid conjugation<sup>63,64</sup>. In soils,  
320 even earthworms can enable such long range movements and increase horizontal gene  
321 transfer at the level of the entire soil matrix<sup>65</sup>. Our findings provide additional insights into  
322 fungal hyphae-mediated dispersal by demonstrating that they cause higher spatial  
323 intermixing of plasmid donors and potential recipients at local scales within a single colony,  
324 which in turn also promotes plasmid conjugation. Thus, fungal hyphae can promote the  
325 spread of plasmid-encoded traits over a range of length scales, from local scales within  
326 individual expanding colonies to longer scales between colonies. Such conclusions are not  
327 limited to antibiotic resistance-encoding plasmids but could be of relevance to a variety of  
328 plasmids, including those important for virulence, environmental remediation, and  
329 biotechnology.

330

331

## 332 **ACKNOWLEDGEMENTS**

333

334 C.R. was supported by grants from the National Natural Science Foundation of China  
335 (42277298) and the 2115 Talent Development Program of the China Agricultural University  
336 (1191-00109012) awarded to G.W. C.R. was also supported by a grant from China  
337 Scholarship Council, State Scholarship fund. J.R. was supported by a grant from the Swiss  
338 National Science Foundation (P2EZP3\_199849) awarded to J.R. and an Eawag Discretionary  
339 Funds grant (category SEED) awarded to D.R.J. G.G. was supported by a grant from the Swiss  
340 National Science Foundation (310030\_188642) awarded to Martin Ackermann. We thank  
341 Professor Liqun Zhang, Department of Plant Pathology, China Agricultural University, for

---

342 providing the *Pseudomonas aeruginosa* PAO1 strains and Dr. Maria Pilar Garcillán-Barcia,  
343 Instituto de Biomedicina y Biotecnología, University of Cantabria, for providing plasmid  
344 pAR145ecfp. We thank Dr. Anne Greet Bittermann, Scientific Center for Optical and Electron  
345 Microscopy (ETH, Zürich, Switzerland) (<https://scopem.ethz.ch/>) for assistance with the  
346 scanning electron microscopy. We thank Miaoxiao Wang, Yinyin Ma, and Deepthi Vinod for  
347 helpful discussions. Finally, we thank Laika, a four-year-old Galgo Español (*Canis familiaris*),  
348 for graciously allowing us to isolate *Penicillium* sp. laika from her paw.

349

350

## 351 **AUTHOR CONTRIBUTIONS**

352

353 C.R. and J.R. contributed equally to this work. C.R., J.R., G.W., and D.R.J. conceived and  
354 developed the research question. C.R., J.R., and D.R.J. designed the experiments. C.R.  
355 performed the experiments. G.G. developed the intermixing index calculation based on  
356 Fourier transforms. C.R., J.R., and G.G. analyzed the data. J.R. and D.R.J. prepared the  
357 manuscript. All authors contributed to the interpretation of the results and gave critical  
358 input to the final version of the manuscript. D.R.J. and G.W. coordinated the study.

359

360

## 361 **DECLARATION OF INTERESTS**

362

363 The authors declare no competing interests.

364

365

## 366 **INCLUSION AND DIVERSITY**

---

367

368 We support inclusive, diverse, and equitable conduct of research.

369

370

371 **MAIN-TEXT FIGURE/TABLE LEGENDS**

372

373 **Figure 1. Experimental system and approach. A,** Our experimental system consists of pairs  
374 of isogenic mutant strains of *P. aeruginosa* or *P. stutzeri*. The competing strains of *P.*  
375 *aeruginosa* PAO1-*gfp* and PAO1-*rfp* and *P. stutzeri* A1601-*egfp* and A1601-*ecfp* can  
376 completely reduce nitrate ( $\text{NO}_3^-$ ) to nitrogen gas ( $\text{N}_2$ ) when grown in an anoxic environment  
377 with an exogenous supply of nitrate. They express different fluorescent proteins but are  
378 otherwise genetically and phenotypically identical. The cross-feeding strains of *P. stutzeri*  
379 A1602-*egfp* and A1603-*ecfp* have single loss-of-function deletions in different steps of the  
380 denitrification pathway that cause them to cross-feed nitrite ( $\text{NO}_2^-$ ) when grown in an anoxic  
381 environment with an exogenous supply of nitrate. Definitions: Nar, nitrate reductase; Nir,  
382 nitrite reductase; Nor, nitric oxide reductase; Nos, nitrous oxide reductase. Thick colored  
383 arrows indicate the metabolic processes performed by each strain and the color of the  
384 respective chromosomally-encoded fluorescent protein that they express (green, red, or  
385 cyan fluorescent protein). **B,** We mixed pairs of the bacterial strains with *Penicillium* sp. laika  
386 and inoculated the mixtures onto the surfaces of nutrient-amended agar plates. We first  
387 incubated the agar plates in oxic conditions to promote the development of a hyphal  
388 network. We then incubated them in anoxic conditions to stop growth of *Penicillium* sp.  
389 laika and allow the bacteria to grow and disperse according to their anoxia-induced  
390 interactions (competition or cross-feeding). We performed control experiments without  
391 *Penicillium* sp. laika in otherwise identical experiments. See also Figure S1.

---

392

393 **Figure 2. Range expansions of competing bacterial strains in the absence or presence of**  
394 **fungus hyphae.** Representative images after four days of range expansion in the **A,B** absence  
395 or **C,D** presence of *Penicillium* sp. laika. Images are for the competing pair of **A,C** *P.*  
396 *aeruginosa* PAO1 or **B,D** *P. stutzeri* A1601 strains. Quantification of the intermixing index  
397 using the local scale Fourier transform method as a function of distance from the centroid  
398 for the competing pair of **E** *P. aeruginosa* PAO1 or **F** *P. stutzeri* A1601 strains. Quantification  
399 of the intermixing index using the intersection method as a function of distance from the  
400 centroid for the competing pair of **G** *P. aeruginosa* PAO1 or **H** *P. stutzeri* A1601 strains For  
401 **E,F,G,H**, we quantified the intermixing index in the radial direction from the outer edge of  
402 the inoculation area to the outer edge of the final expansion frontier at the end of the range  
403 expansion experiment. The insets depict the intermixing indices at the expansion frontier  
404 (35  $\mu\text{m}$  band from the expansion edge). This intermixing index is the sum of indices at  
405 increments of 5  $\mu\text{m}$  across the 35  $\mu\text{m}$ -wide frontier. The lines are the moving averages of  
406 the intermixing index. Each data point is the measurement for an independent experimental  
407 replicate ( $n = 5$ ) and  $P$  is for a two-sample two-sided Welch test. See also Figures S2-S5.

408

409 **Figure 3. Range expansions of cross-feeding bacterial strains in the absence or presence of**  
410 **fungus hyphae.** Representative images of the cross-feeding pair of *P. stutzeri* A1602 and  
411 A1603 strains after four days of range expansion in the **A** absence or **B** presence of  
412 *Penicillium* sp. laika. **C**, Quantification of the intermixing index using the local scale Fourier  
413 transform method as a function of distance from the centroid. **D**, Quantification of the  
414 intermixing index using the intersection method as a function of distance from the centroid.  
415 For **C,D**, we quantified the intermixing index in the radial direction from the outer edge of  
416 the inoculation area to the outer edge of the final expansion frontier at the end of the range

---

417 expansion experiment. The inset depicts the intermixing index at the expansion frontier (35  
418  $\mu\text{m}$  band from the expansion edge). This intermixing index is the sum of indices at  
419 increments of 5  $\mu\text{m}$  across the 35  $\mu\text{m}$ -wide frontier. The lines are the moving averages of  
420 the intermixing index. Each data point is the measurement for an independent experimental  
421 replicate ( $n = 5$ ) and  $P$  is for a two-sample two-sided Welch test. See also Figures S2-S5.

422

423 **Figure 4. Effect of defects in pili and flagellum-mediated motility on the extent of range**  
424 **expansion in the presence of fungal hyphae.** Representative images of **A** the ancestral *P.*  
425 *aeruginosa* PAO1-*rfp* strain with functional flagella and pili, **B** the PAO1- $\Delta$ *pilA*-*rfp* strain that  
426 is defective in pili-mediated motility, and **C** the PAO1- $\Delta$ *flhC*-*rfp* strain that is defective in  
427 flagellum-mediated motility after four days of range expansion. We mixed each bacterial  
428 strain individually with *Penicillium* sp. laika and inoculated them onto a separate nutrient-  
429 amended agar plate. **D**, Quantification of the total expansion radius for each bacterial strain.  
430 Each data point is the measurement for an independent experimental replicate ( $n = 5$ ) and  $P$   
431 is for a two-sample two-sided Welch test with a Holm-Bonferroni correction. For **E,F,G**, we  
432 used a glass fiber with a diameter of 5  $\mu\text{m}$  as an abiotic surrogate for fungal hyphae.  
433 Representative confocal laser scanning microscopy images are for **E** the ancestral *P.*  
434 *aeruginosa* PAO1-*rfp* strain with a functional flagellum and pili, **F** the PAO1- $\Delta$ *pilA*-*rfp* strain  
435 that is defective in pili-mediated motility, and **G** the PAO1- $\Delta$ *flhC*-*rfp* strain that is defective in  
436 flagellum-mediated motility after four days of range expansion.

437

438 **Figure 5. Range expansions of interacting bacterial strains along a glass fiber.** We used a  
439 glass fiber with a diameter of 5  $\mu\text{m}$  as an abiotic surrogate for fungal hyphae, thus allowing  
440 us to exclude potential biotic interactions that may affect dispersal abilities and intermixing.  
441 Representative confocal laser scanning microscopy images are for **A** the competing pair of *P.*

---

442 *stutzeri* A1601 strains, and **B** the cross-feeding pair of *P. stutzeri* A1602 and A1603 strains.  
443 Note that both strains rapidly co-migrated along the glass fiber regardless of the interaction  
444 imposed between them.

445

446 **Figure 6. Range expansions of pairs of *P. aeruginosa* PAO1- $\Delta$ fliC strains in the absence or**  
447 **presence of fungal hyphae.** Representative images after four days of range expansion in the  
448 **A** absence or **B** presence of *Penicillium* sp. laika. White circles depict the inoculation area  
449 (inner) and a fixed distance within the expansion region where spatial intermixing was  
450 clearly affected by fungal hyphae (outer). **C**, We quantified the intermixing index using the  
451 local scale Fourier transform method at radial increments of 5  $\mu$ m between the outer edge  
452 of the inoculation area (inner circle) and the outer edge of the expansion area (outer circle).  
453 We corrected each measurement by the circumference at which it was measured and  
454 summed all the indices across the expansion area for each replicate. Each data point is a  
455 measurement for an independent experimental replicate (n = 3) and *P* is for a two-sample  
456 two-sided Welch test. **D**, Scanning electron microscopy images. Note that the bacteria  
457 occupy the surface of the hyphae as well as the interstices between them.

458

459 **Figure 7. Plasmid conjugation between competing bacterial strains during range**  
460 **expansion in the absence or presence of fungal hyphae.** Representative images of the  
461 competing pair of *P. stutzeri* A1601 strains after four days of range expansion in the **A,B**  
462 absence or **C,D** presence of *Penicillium* sp. laika. In this system, *P. stutzeri* A1601-*egfp*  
463 carried plasmid pAR145 and was the plasmid donor strain while *P. stutzeri* A1601-*ech* was  
464 the potential recipient strain. pAR145 encodes for chloramphenicol resistance and cyan  
465 fluorescent protein. Thus, regions in blue within the expansion area indicate pAR145  
466 presence. **A,C**, Composite images of the green, red and blue channels. **B,D**, Images of only



---

467 the green and blue channels, which aids in the visualization of transconjugants. Note that  
468 we increased the intensity of the green channel, which caused plasmid donors to appear as  
469 only green and improved visual contrast between plasmid donors and transconjugants. **E**,  
470 Number of transconjugants relative to the total number of potential recipients across the  
471 entire area. **F**, Integrated number of transconjugants relative to the expansion  
472 circumference at a given radius over the leading 350  $\mu\text{m}$ -radial region of the expansion area,  
473 which corresponds to the expansion region after spatial segregation of the strains in the  
474 absence of fungal hyphae. We chose this distance because it corresponds to the area where  
475 the hyphal network clearly influences spatial intermixing via bacterial dispersal. For **E,F**, each  
476 data point is the measurement for an independent experimental replicate ( $n = 5$ ) and  $P$  is for  
477 a two-sample two-sided Welch test.

478

479

## 480 **STAR METHODS**

481

### 482 **RESOURCE AVAILABILITY**

483

#### 484 **Lead contact**

485 Further information and requests for resources, reagents and microbial strains should be  
486 directed to and will be fulfilled by the lead contacts David R. Johnson  
487 ([david.johnson@eawag.ch](mailto:david.johnson@eawag.ch)) and Gang Wang ([gangwang@cau.edu.cn](mailto:gangwang@cau.edu.cn)).

488

#### 489 **Materials availability**

490 All fungal and bacterial strains generated in this study are available from the lead contacts  
491 with a completed Materials Transfer Agreement.

---

492

493 **Data and code availability.**

494 All data and code have been deposited in the Eawag Research Data Institutional Repository  
495 (<https://opendata.eawag.ch/>) and are publically available as of the date of publication at the  
496 following DOI: (<https://doi.org/10.25678/0007GJ>).

497

498

499 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

500

501 **Microbial strains**

502 We used isogenic mutants of *P. stutzeri* A1601<sup>15,50</sup> and *P. aeruginosa* PAO1 to test the  
503 effects of fungal hyphae on the maintenance of bacterial diversity during range expansion.  
504 We assembled these strains into three pairs. The first pair consisted of *P. stutzeri* A1601-  
505 *egfp* and A1601-*ecfp* (Figure 1A and Table S1). Both of these strains have the complete  
506 denitrification pathway and, aside from having different chromosomally-located fluorescent  
507 protein-encoding genes, are genetically identical<sup>15,50</sup>. They thus compete with each other  
508 when grown together in an anoxic environment with nitrate (NO<sub>3</sub><sup>-</sup>) as the growth-limiting  
509 resource. The second pair consisted of *P. stutzeri* A1602-*egfp* and A1603-*ecfp* (Figure 1A and  
510 Table S1). Strain A1602-*egfp* has a loss-of-function deletion in the nitrate reductase-  
511 encoding *narG* gene while strain A1603-*ecfp* has a loss-of-function deletion in the nitrite  
512 (NO<sub>2</sub><sup>-</sup>) reductase-encoding *nirS* gene<sup>50</sup>. They therefore engage in a nitrite cross-feeding  
513 interaction when grown together in an anoxic environment with nitrate as the growth-  
514 limiting nutrient<sup>50</sup>. All the *P. stutzeri* strains also have a loss-of-function deletion in the *comA*  
515 gene that prevents recombination when grown together<sup>50,71</sup> and a chromosomally-located  
516 gentamycin resistance gene to prevent contamination during experiments<sup>66</sup>. All the *P.*

---

517 *stutzeri* strains have a chromosomally-located isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG)-  
518 inducible fluorescent protein-encoding gene that encodes for either cyan or green  
519 fluorescent protein<sup>15,66</sup>, which enables us to distinguish them by fluorescence microscopy  
520 when grown together. A complete description of the strains, along with details of their  
521 genetic construction, are reported in detail elsewhere<sup>15,50,66</sup>. The third pair consisted of *P.*  
522 *aeruginosa* PAO1-*gfp* and PAO1-*rfp* (Figure 1A and Table S1). Strain PAO1-*gfp* carries  
523 plasmid pSMC21 that contains the green fluorescent protein-encoding *gfp* gene while strain  
524 PAO1-*rfp* carries plasmid pBRM that contains the red fluorescent protein-encoding *rfp* gene  
525 (Table S1). As with the *P. stutzeri* A1601 strains, both *P. aeruginosa* PAO1 strains have the  
526 complete denitrification pathway and, aside from carrying different plasmid-located  
527 fluorescent protein-encoding genes, are genetically identical. They therefore also compete  
528 with each other when grown together in an anoxic environment amended with nitrate as  
529 the growth-limiting resource. We routinely grew all the *P. stutzeri* and *P. aeruginosa* strains  
530 with lysogeny broth (LB) medium at 30°C.

531

532 We used the hyphae-forming fungus *Penicillium* sp. laika to test the effects of fungal hyphae  
533 on the maintenance of bacterial diversity during range expansion. We isolated this strain  
534 from the paw of a Galgo Español (*Canis familiaris*) by physical contact with an LB agar plate  
535 supplemented with 50  $\mu\text{g ml}^{-1}$  kanamycin. After incubation of the LB agar plate for three  
536 days at 20°C, we obtained a white villiform fungal colony and purified the colony by  
537 streaking a second time on an LB agar plate supplemented with 50  $\mu\text{g ml}^{-1}$  kanamycin. We  
538 routinely grew *Penicillium* sp. laika in liquid LB medium at 20°C. We determined the  
539 taxonomic affiliation of *Penicillium* sp. laika by Sanger sequencing of a PCR-amplified 520 bp  
540 fragment of the internal transcribed spacer region (primers: ITS1 5'-  
541 TCCGTAGGTGAACCTGCGG-3'; ITS4 5'-TCCTCCGCTTATTGATATGC-3')<sup>72</sup>. We submitted the

---

542 consensus sequence to the UNITE database<sup>69</sup> and queried for similar sequences using the  
543 BLAST algorithm (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The alignment has 100%  
544 sequence coverage and 100% sequence identity to GenBank accessions MG818940.1,  
545 KT270333.1, KM396384.1, and KM396380.1 assigned to the *Penicillium glabrum/thomii*  
546 group. We summarized the morphological characteristics of *Penicillium* sp. laika in Table S1.

547

## 548 **METHOD DETAILS**

549

550 *Experimental procedure to test the effects of fungal hyphae on bacterial range expansion.*

551

552 To prepare *Penicillium* sp. laika for experimentation, we first grew the strain on oxyc LB agar  
553 plates for five days to allow for spore maturation. We then removed the fungal spores from  
554 the plate using a sterile inoculation loop and transferred the spores to 1 mL of oxyc 0.9%  
555 (w/v) sodium chloride solution. We suspended the spores by vortexing for 10 minutes and  
556 adjusted the optical density at 600 nm (OD<sub>600</sub>) to 1. To prepare the bacterial strains for  
557 experimentation, we first grew each strain separately overnight in oxyc LB medium at  
558 37°C. After reaching stationary phase, we adjusted the densities of each culture to an OD<sub>600</sub>  
559 of 2, centrifuged the cultures at 3600 x g for 5 min at room temperature, discarded the  
560 supernatants, and suspended the cells in 1 mL of oxyc 0.9% (w/v) sodium chloride solution.  
561 We then mixed the corresponding bacterial strains together at equal initial proportions and  
562 diluted the bacterial mixtures to approximately 10<sup>6</sup> colony forming units ml<sup>-1</sup> in 0.9% (w/v)  
563 sodium chloride solution.

564

565 We performed range expansion experiments using a modified version of a protocol  
566 described in detail elsewhere<sup>15,20</sup>. Briefly, for experiments with pairs of *P. stutzeri* or *P.*

---

567 *aeruginosa* strains, we mixed equal volumes of the fungal and bacterial solutions and  
568 deposited a single 2  $\mu$ l droplet onto the middle of a modified oxic LB agar plate. The  
569 modified LB agar plate contained 10 g L<sup>-1</sup> tryptone, 5 g L<sup>-1</sup> yeast extract, 10 g L<sup>-1</sup> sodium  
570 chloride, 20 g L<sup>-1</sup> agar, 20 mM sodium nitrate (NO<sub>3</sub><sup>-</sup>), and 100  $\mu$ M IPTG. We then incubated  
571 the LB agar plates in oxic conditions for two days at 20°C to allow *Penicillium* sp. laika to  
572 form a dense hyphal network that extends beyond the bacterial expansion range (Figure  
573 S1). We note that the cross-feeding bacterial pair engages in a competitive interaction for  
574 oxygen under oxic conditions and generates patterns consistent with those generated by  
575 the competing pair under the same condition<sup>15,73</sup>. We then transferred the plates into a  
576 glove box (Coy Laboratory Products, Grass Lake, MI) filled with an anoxic nitrogen  
577 (N<sub>2</sub>):hydrogen (H<sub>2</sub>) (97%:3%) atmosphere at 20°C. After incubation in anoxic conditions for  
578 two additional days, which stopped growth of *Penicillium* sp. laika and promoted the anoxia-  
579 dependent interactions between the bacterial strains (competition or cross-feeding), the  
580 bacterial consortia had expanded across the fungal network to near the network's edge but  
581 without surpassing it. The intermixing indices measured at the expansion frontier therefore  
582 correspond to the anoxic growth period. We then removed the LB agar plates from the  
583 glove box and exposed them to ambient air for 1 h to promote maturation of the IPTG-  
584 inducible fluorescent proteins. We performed all experiments with five experimental  
585 replicates.

586

587 *Experimental procedure used to test the effects of active motility on bacterial range*  
588 *expansion.*

589

590 To test the mechanism driving bacterial dispersal along fungal hyphae, we performed range  
591 expansion experiments using *P. aeruginosa* PAO1-derived mutants that carry plasmid pBRM

---

592 but either cannot generate functional type IV pili (strain PAO1- $\Delta$ *pilA-rfp*) or a functional  
593 flagellum (strain PAO1- $\Delta$ *fliC-rfp*) (Table S1). We additionally used the ancestral strain PAO1-  
594 *rfp* as a control. The experimental procedures are identical to those described above except  
595 that we mixed each strain individually with *Penicillium* sp. laika (i.e., these experiments  
596 contained only a single bacterial strain). We performed all experiments with five  
597 experimental replicates.

598

599 *Experimental procedure used to test for possible topographical effects caused by the fungal*  
600 *hyphae.*

601

602 To test whether the presence of fungal hyphae could create topographical effects that affect  
603 the maintenance of diversity during range expansion, we performed range expansion  
604 experiments using pairs of *P. aeruginosa* PAO1- $\Delta$ *fliC-rfp* and PAO1- $\Delta$ *fliC-gfp*. PAO1- $\Delta$ *fliC-gfp*  
605 carries plasmid pSMC21 that encodes for kanamycin resistance and green fluorescent  
606 protein (Table S1). The experimental procedures are identical to those described above. We  
607 performed all experiments with five experimental replicates.

608

609 *Experimental procedure used to test the effects of fungal hyphae on plasmid conjugation.*

610

611 To test whether fungal hyphae affect plasmid conjugation during range expansion, we  
612 introduced plasmid pAR145ecfp, which encodes for chloramphenicol resistance and cyan  
613 fluorescent protein, into *P. stutzeri* A1601-*egfp* by conjugation from the plasmid donor  
614 strain *Escherichia coli* DH5 $\alpha$  using conventional filter mating. This plasmid encodes for  
615 chloramphenicol resistance and cyan fluorescent protein and is self-transmissible (Table S1).  
616 We then quantified the extent of pAR145ecfp conjugation during range expansion in the

---

617 absence or presence of fungal hyphae using the same strains and procedures as described  
618 above. We quantified the number of transconjugants that emerged during range expansion  
619 from confocal laser scanning microscopy (CLSM) images as described below. We performed  
620 all experiments with five experimental replicates.

621

622 *Confocal laser scanning microscopy.*

623

624 After completion of the range expansion experiments, we imaged the expansions directly on  
625 the agar plates without physically disturbing them using a Leica TCS SP5 II confocal laser  
626 scanning microscope (Leica Microsystems, Wetzlar, Germany) with a 5x HCX FL air  
627 immersion lens, a numerical aperture of 0.12, a frame size of 1024 × 1024, and a pixel size of  
628 3.027 μm. We set the laser to 458 nm for the excitation of cyan fluorescent protein, to  
629 488 nm for the excitation of green fluorescent protein, and to 514 nm for the excitation of  
630 red fluorescent protein.

631

632 *Scanning electron microscopy (SEM).*

633

634 To perform SEM imaging of fungal-bacterial consortia, we first vapor fixed the consortia  
635 with 2.5% electron microscopy grade glutaraldehyde and 2% osmium tetroxide (OsO<sub>4</sub>) in  
636 distilled water. We then exposed the samples to glutaraldehyde for 90 minutes followed by  
637 OsO<sub>4</sub> for another 90 minutes. We next excised the vapor-fixed colonies from the plate, dried  
638 them in ambient air, and mounted the samples with conductive carbon cement onto SEM  
639 aluminium stubs. After outgasing overnight, we coated the samples with a 5 nm thick layer  
640 of platinum/palladium with rotation in a Safematic CCU-010 Metal Sputter Coater (LabTech  
641 Inc., Hopkinton, MA, USA). Finally, we imaged the samples with a Shottky Field Emission

---

642 Scanning Electron Microscope SU5000 (Hitachi High-Tech, Tokyo, Japan) at 2kV by  
643 secondary electron detection in collaboration with the Scientific Center for Optical and  
644 Electron Microscopy (ETH, Zürich, Switzerland) (<https://scopem.ethz.ch>).

645

## 646 **Quantification and statistical analyses**

647

648 *Quantification of spatial intermixing.*

649

650 We quantified the magnitude of spatial intermixing (referred to as the intermixing index)  
651 between bacterial populations from the CLSM images<sup>15,20</sup>. The intermixing index provides a  
652 proxy measure of the number of individuals that emigrate from the inoculation area and  
653 contribute to active range expansion<sup>7</sup>. It can therefore be viewed as a proxy measure of  
654 diversity<sup>15,20</sup>. Briefly, if the initial population contains standing genetic diversity, then larger  
655 intermixing indices correspond with higher amounts of that initial standing genetic diversity  
656 that contribute to active range expansion.

657

658 An important challenge of analyzing spatial intermixing in range expansion experiments is to  
659 conserve as much information as possible. Loss of information derives from thresholding of  
660 images, which is necessary to count the number of transitions from one color to another. To  
661 minimize the loss of information, we developed a novel method that applies Fourier  
662 transforms across concentric rings at different expansion radii (Figure S6). This method does  
663 not binarize the data and conserves pixel-level signal intensities. We did this as follows.  
664 Starting with the original CLSM image (Figure S6A), we first extracted the layers that  
665 captured the strains expressing a given fluorescent protein (Figure S6B). We then extracted  
666 1-pixel-wide rings at 3-pixel radial increments (Figure S6C) and transformed each ring into a



---

667 sequence of  $\{\theta_i, px_i\}$ , where  $px_i$  is the value of the pixel that makes an angle of  $\theta_i$  with the  
668 positive x-axis direction (Figure S6D). We calculated the angles from the positive x-direction  
669 that originates at the center of the image and extends in the right direction. To  
670 accommodate for the circular periodicity of the data, we copied the data twice, shifted the  
671 values of the angles by  $2*\pi$  and  $4*\pi$ , and appended it to the original sequence. The length of  
672 the final sequence was therefore three times longer than the original one. We then  
673 performed Fourier transforms on the final sequence, whereby the resulting frequencies  
674 correspond to the inverse of angles (Figure S6E). Each data point can be understood as how  
675 much mixing (Fourier amplitude) occurs with the corresponding frequency. In order to  
676 obtain the intermixing index at various length scales, we integrated the area under the  
677 curve of the Fourier transforms (Figure S6F). The dark grey area corresponds to intermixing  
678 at global scales (5 to 50 degrees), the blue area corresponds to intermixing at intermediate  
679 scales (0.5 to 5 degrees) and the red area corresponds to intermixing at local scales (0.2 to  
680 0.5 degrees) (Figure S6F). We used local scales in this study because these scales match the  
681 pixel sizes at which we observed the finest scales of intermixing of different strains in our  
682 experiments.

683

684 In parallel, we also quantified the intermixing index for all of our experiments using a well-  
685 established intersection method<sup>13</sup>. To achieve this, we used a circular windowing approach  
686 to quantify the number of intersections between populations using Fiji (v1.53c) plugins  
687 (<https://fiji.sc>). Briefly, we first thresholded one of the color channels using the Huang  
688 algorithm implemented in ImageJ (<https://imagej.net>) and removed it from the image. We  
689 then removed remaining noise using the 'remove outliers' method (radius = 5, threshold =  
690 50, bright). We next used the remaining 1-color image as an input to the Sholl plugin of  
691 ImageJ to calculate the number of intersections between background and information-

---

692 containing parts of the image at 5  $\mu\text{m}$  increments from the outer edge of the inoculation  
693 area to the outer edge of the expansion frontier. For a measured number of intersections at  
694 a given radius ( $N_r$ ), we quantified the intermixing index ( $I_r$ ) as:

$$I_r = \frac{N_r}{\pi r / 2}$$

695  
696  
697 We provided all of the intermixing indices calculated with the intersection method in the  
698 main figures or in Figure S3. Note that the intersection method resulted in the same  
699 qualitative conclusions as the local scale Fourier transform method.

700

701 For both the local scale Fourier transform and the intersection method, we accounted for  
702 unequal expansion sizes between biological replicates and treatments by transforming the  
703 radii to a relative scale (maximum radius set to one). After inspection of the trends of the  
704 intermixing index along the expansion radii, we removed the intermixing indices from the  
705 leading 2% of the expansion areas for all range expansions due to inadequate focus. Briefly,  
706 the thickness of the biomass becomes thinner towards the expansion frontier, which causes  
707 us to lose focus. We then defined the expansion frontier (*i.e.*, the actively growing layer of  
708 cells at the expansion edge) as a 35  $\mu\text{m}$  wide band at the expansion edge based on  
709 experimental measurements reported in a similar study<sup>19</sup>. This width corresponds to  $\sim 12$   
710 cells assuming an average cell length of 2-3  $\mu\text{m}$ . The reported intermixing indices are the  
711 sum of the circumference-corrected intermixing indices at 5  $\mu\text{m}$  radial increments within the  
712 35  $\mu\text{m}$  wide band.

713

714 *Quantification of plasmid pAR145ecfp transconjugants during range expansion.*

715

---

716 We quantified the number of transconjugants (*i.e.*, recipients that acquired plasmid  
717 pAR145ecfp) from the CLSM images. We first used functions implemented in Fiji (*v1.53c*)  
718 (<https://fiji.sc>) as described above for image preprocessing. We then counted the total  
719 number of overlapping blue and red pixels at 5  $\mu\text{m}$  radial increments from the outer edge of  
720 the inoculation area to the outer edge of the expansion frontier. We followed the same  
721 procedures to quantify the number of blue pixels only. We then divided the number of  
722 overlapping blue and red pixels (*i.e.* the number of transconjugants) by the number of blue  
723 pixels (*i.e.* the total number of potential recipients) at each radial increment. We next  
724 selected a radius of 350  $\mu\text{m}$  from the expansion edge as the region to statistically test the  
725 effects of fungal hyphae on the number of transconjugants. This is because this radius  
726 corresponds to the area where the effects of fungal hyphae on spatial mixing are  
727 quantifiable. We further estimated the total number of transconjugants in each range  
728 expansion by selective plating on LB agar plates amended with 30  $\mu\text{g ml}^{-1}$  chloramphenicol.  
729 For image presentation, we increased the intensity of the green channel. This caused the  
730 plasmid donors to appear as green and improved their visual differentiation from  
731 transconjugants.

732

### 733 *Quantification of biomass.*

734

735 We quantified the total biomass of individual range expansions by flow cytometry. We first  
736 used a spatula to detach and transfer the biomass from an entire range expansion into a 50  
737 ml centrifuge tube containing 20 ml of phosphate-buffered saline solution and 1%  
738 potassium citrate. We then vortexed the solution for 10 minutes to fully suspend the cells  
739 and diluted the solution by 1000x (v:v). We next transferred 500  $\mu\text{L}$  of the solution into a 3.5  
740 mL tube, added 5  $\mu\text{L}$  of SYBR Green, and incubated the tube in the dark for 10 min at 37°C.

---

741 We then quantified the number of SYBR Green-labeled cells using a Accuri C6 flow  
742 cytometer (BD Accuri, San Jose, CA, USA) equipped with a 50 mW laser emitting at a fixed  
743 wavelength of 488 nm<sup>74</sup>. The flow cytometer was equipped with volumetric counting  
744 hardware and calibrated to measure the number of particles in a 50  $\mu$ L volume. We  
745 processed all data with the Accuri CFlow software (BD Accuri, San Jose, CA, USA) with  
746 electronic gating to separate bacterial-derived signals from instrument noise and sample  
747 background.

748

749 *Statistical analyses.*

750

751 We performed all statistical tests in the R software environment<sup>70</sup>. For each dataset, we  
752 tested for homoscedasticity with the Bartlett test and normality with the Wilk-Shapiro test.  
753 We assessed statistical significance between means of the fungal hyphae “Presence” and  
754 “Absence” factor levels using two-sample two-sided Welch tests implemented with the R  
755 core function *t.test* with unequal variances. We chose the Welch test because none of our  
756 datasets significantly deviated from normality but some significantly deviated from  
757 homoscedasticity. We reported the statistical test and the sample size for each test in the  
758 results section.

759

---

760 **REFERENCE LIST**

- 761 1. Hall-Stoodley, L., Costerton, J.W., and Stoodley, P. (2004). Bacterial biofilms: From the  
762 natural environment to infectious diseases. *Nat. Rev. Microbiol.* *2*, 95-108.
- 763 2. Flemming, H.C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S.A., and Kjelleberg, S.  
764 (2016). Biofilms: An emergent form of bacterial life. *Nat. Rev. Microbiol.* *14*, 563-575.
- 765 3. Man, W.H., de Steenhuijsen Pipers, W.A.A., and Bogaert, D. (2017) The microbiota of the  
766 respiratory tract: gatekeeper to respiratory health. *Nat Rev. Microbiol.* *15*, 259-270.
- 767 4. Tilman, D., and Kareiva, P.M. (1997). *Spatial ecology: The role of space in population*  
768 *dynamics and interspecific interactions (MPB-30)* (Princeton University Press).
- 769 5. Excoffier, L., Foll, M., and Petit, R.J. (2008). Genetic consequences of range expansions.  
770 *Annu. Rev. Ecol. Evol. Syst.* *40*, 481-501.
- 771 6. Golding, I., Cohen, I., and Ben-Jacob, E. (1999). Studies of sector formation in expanding  
772 bacterial colonies. *Europhys. Lett.* *48*, 587-593.
- 773 7. Hallatschek, O., Hersen, P., Ramanathan, S., and Nelson, D.R. (2007). Genetic drift at  
774 expanding frontiers promotes gene segregation. *Proc. Natl. Acad. Sci. U. S. A.* *104*,  
775 19926-19930.
- 776 8. Hallatschek, O., and Nelson, D.R. (2010). Life at the front of an expanding population.  
777 *Evol.* *64*, 193-206.
- 778 9. Korolev, K.S., Müller, M.J.I., Karahan, N., Murray, A.W., Hallatschek, O., and Nelson, D.R.  
779 (2012). Selective sweeps in growing microbial colonies. *Phys. Biol.* *9*, 026008.
- 780 10. Eckburg, P.B, Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S.R.,  
781 Nelson, K.E., and Relman, D.A. (2005). Diversity of the human intestinal microbial flora.  
782 *Science* *308*, 1635-1638.
- 783 11. Gans, J., Wolinsky, M., and Dunbar, J. (2005). Computational improvements reveal great  
784 bacterial diversity and high metal toxicity in soil. *Science* *309*, 1387-1390.

- 
- 785 12. The Human Microbiome Project Consortium. (2012). Structure, function and diversity of  
786 the healthy human microbiome. *Nature* 486, 207-214.
- 787 13. Momeni, B., Brileya, K.A., Fields, M.W., and Shou, W.Y. (2013). Strong inter-population  
788 cooperation leads to partner intermixing in microbial communities. *Elife* 2, e00230.
- 789 14. Müller, M.J.I., Neugeboren, B.I., Nelson, D.R., and Murray, A.W. (2014). Genetic drift  
790 opposes mutualism during spatial population expansion. *Proc. Natl. Acad. Sci. U. S. A.*  
791 111, 1037-1042.
- 792 15. Goldschmidt, F., Regoes, R.R., and Johnson, D.R. (2017). Successive range expansion  
793 promotes diversity and accelerates evolution in spatially structured microbial  
794 populations. *ISME J.* 11, 2112-2123.
- 795 16. Hibbing, M.E., Fuqua, C., Parsek, M.R., and Peterson, S.B. (2010). Bacterial competition:  
796 surviving and thriving in the microbial jungle. *Nat. Rev. Microbiol.* 8, 15-25.
- 797 17. Foster, K.R., and Bell, T. (2012). Competition, not cooperation, dominates interactions  
798 among culturable microbial species. *Curr. Biol.* 22, 1845-1850.
- 799 18. Palmer, J.D., and Foster, K.R. (2022). Bacterial species rarely work together. *Science* 376,  
800 581-582.
- 801 19. Mitri, S., Clarke, E., and Foster, K.R. (2016). Resource limitation drives spatial  
802 organization in microbial groups. *ISME J.* 10, 1471-1482.
- 803 20. Goldschmidt, F., Regoes, R.R., and Johnson, D.R. (2018). Metabolite toxicity slows local  
804 diversity loss during expansion of a microbial cross-feeding community. *ISME J.* 12, 136-  
805 144.
- 806 21. van Gestel, J., Weissing, F.J., Kuipers, O.P., and Kovács, A.T. (2014). Density of founder  
807 cells affects spatial pattern formation and cooperation in *Bacillus subtilis* biofilms. *ISME*  
808 *J.* 8, 2069-79.

- 
- 809 22. Goldschmidt, F., Caduff, L., and Johnson, D.R. (2021). Causes and consequences of  
810 pattern diversification in a spatially self-organizing microbial community. *ISME J.* *15*,  
811 2415-2426.
- 812 23. Eigentler, L., Kalamara, M., Ball, G., MacPhee, C.E., Stanley-Wall, N.R., Davidson, F.A.  
813 (2022). Founder cell configuration drives competitive outcome within colony biofilms.  
814 *ISME J.* *16*, 1512-1522.
- 815 24. Ciccarese, D., Zuidema, A., Merlo, V., and Johnson, D.R. (2020). Interaction-dependent  
816 effects of surface structure on microbial spatial self-organization. *Philos. Trans. R. Soc.*  
817 *Lond. B Biol. Sci.* *375*, 20190246.
- 818 25. Carson, J.K., Gonzalez-Quiñones, V., Murphy, D.V., Hinz, C., Shaw, J.A., and Gleeson, D.B.  
819 (2010). Low pore connectivity increases bacterial diversity in soil. *Appl. Environ.*  
820 *Microbiol.* *76*, 3936-3942.
- 821 26. de Boer, W., Folman, L.B., Summerbell, R.C., and Boddy, L. (2005). Living in a fungal  
822 world: Impact of fungi on soil bacterial niche development. *FEMS Microbiol. Rev.* *29*,  
823 795-811.
- 824 27. Hogan, D.A., and Kolter, R. (2002). *Pseudomonas-Candida* interactions: An ecological  
825 role for virulence factors. *Science* *296*, 2229-2232.
- 826 28. Underhill, D.M., and Lliev, L.D. (2014). The mycobiota: Interactions between commensal  
827 fungi and the host immune system. *Nat. Rev. Immunol.* *14*, 405-416.
- 828 29. van Overbeek, L.S., and Saikkonen, K. (2016). Impact of bacterial-fungal interactions on  
829 the colonization of the endosphere. *Trends Plant Sci.* *21*, 230-242.
- 830 30. Tipton, L., Müller C.L., Kurtz, Z.D., Huang, L., Kleerup, E., Morris, A., Bonneau, R., and  
831 Ghedin, E. (2018). Fungi stabilize connectivity in the lung and skin microbial ecosystems.  
832 *Microbiome* *6*, 12.

- 
- 833 31. Frey-Klett, P., Burlinson, P., Deveau, A., Barret, M., Tarkka, M., and Sarniguet, A. (2011).  
834 Bacterial-fungal interactions: Hyphens between agricultural, clinical, environmental, and  
835 food microbiologists. *Microbiol. Mol. Biol. Rev.* 75, 583-609.
- 836 32. Kohlmeier, S., Smits, T.H.M., Ford, R.M., Keel, C., Harms, H., and Wick, L.Y. (2005). Taking  
837 the fungal highway: Mobilization of pollutant-degrading bacteria by fungi. *Environ. Sci.*  
838 *Technol.* 39, 4640-4646.
- 839 33. Warmink, J.A., Nazir, R., Corten, B., and van Elsas, J.D. (2011). Hitchhikers on the fungal  
840 highway: The helper effect for bacterial migration via fungal hyphae. *Soil Biol. Biochem.*  
841 43, 760-765.
- 842 34. Pion, M., Bshary, B., Bindschedler, S., Filippidou, S., Wick, L.Y., Job, D., and Junier, P.  
843 (2013). Gains of bacterial flagellar motility in a fungal world. *Appl. Environ. Microbiol.* 79,  
844 6862-6867.
- 845 35. Worrlich, A., König, S., Miltner, A., Banitz, T., Centler, F., Frank, K., Thullner, M., Harms,  
846 H., Kästner, M., and Wick, L.Y. (2016). Mycelium-like networks increase bacterial  
847 dispersal, growth, and biodegradation in a model ecosystem at various water potentials.  
848 *Appl. Environ. Microbiol.* 82, 2902-2908.
- 849 36. Zhang, Y.C., Kastman, E.K., Guasto, J.S., and Wolfe, B.E. (2018). Fungal networks shape  
850 dynamics of bacterial dispersal and community assembly in cheese rind microbiomes.  
851 *Nat. Comm.* 9, 336.
- 852 37. Xiong, B.J., Kleinstüber, S., Sträuber, H., Dusny, C., Harms, H., and Wick, L.Y. (2022)  
853 Impact of fungal hyphae on growth and dispersal of obligate anaerobic bacteria in  
854 aerated habitats. *mBio* 13, e0076922.
- 855 38. Wick, L.Y., Remer, R., Würz, B., Reichenbach, J., Braun, S., Schäfer, F., and Harms, H.  
856 (2007). Effect of fungal hyphae on the access of bacteria to phenanthrene in soil.  
857 *Environ. Sci. Technol.* 41, 500-505.



- 
- 858 39. Furuno, S., Pätzolt, K., Rabe, C., Neu, T.R., Harms, H., and Wick, L.Y. (2010). Fungal  
859 mycelia allow chemotactic dispersal of polycyclic aromatic hydrocarbon-degrading  
860 bacteria in water-unsaturated systems. *Environ. Microbiol.* *12*, 1391-1398.
- 861 40. Banitz, T., Fetzner, I., Johst, K., Wick, L.Y., Harms, H., and Frank, K. (2011). Assessing  
862 biodegradation benefits from dispersal networks. *Ecol. Modell.* *222*, 2552-2560.
- 863 41. Berthold, T., Centler, F., Hübschmann, T., Remer, R., Thullner, M., Harms, H., and Wick,  
864 L.Y. (2016). Mycelia as a focal point for horizontal gene transfer among soil bacteria. *Sci.*  
865 *Rep.* *6*, 36390.
- 866 42. Otto, S., Bruni, E.P., Harms, H., and Wick, L.Y. (2017). Catch me if you can: dispersal and  
867 foraging of *Bdellovibrio bacteriovorus* 109J along mycelia. *ISME J.* *11*, 386-393.
- 868 43. You, X., Kallies, R., Kühn, I., Schmidt, M., Harms, H., Chatzinotas, A., and Wick, L.Y.  
869 (2022). Phage co-transport with hyphal-riding bacterial fuels bacterial invasion in a  
870 water-unsaturated microbial model system. *ISME J.* *16*, 1275-1283.
- 871 44. Tilman, D. (1994). Competition and biodiversity in spatially structured habitats. *Ecol.* *75*,  
872 2-16.
- 873 45. Nadell, C.D., and Bassler, B.L. (2011). A fitness trade-off between local competition and  
874 dispersal in *Vibrio cholerae* biofilms. *Proc. Natl. Acad. Sci. U. S. A.* *108*, 14181-14185.
- 875 46. Datta, M.S., Korolev, K.S., Cvijovic, I., Dudley, C., and Gore, J. (2013). Range expansion  
876 promotes cooperation in an experimental microbial metapopulation. *Proc. Natl. Acad.*  
877 *Sci. U. S. A.* *110*, 7354-7359.
- 878 47. Yawata, Y., Cordero, O.X., Menolascina, F., Hehemann, J.H., Polz, M.F., and Stocker, M.  
879 (2014). Competition-dispersal tradeoff ecologically differentiates recently speciated  
880 marine bacterioplankton populations. *Proc. Natl. Acad. Sci. U. S. A.* *111*, 5622-5627.
- 881 48. Gude, S., Pinçe, E., Taute, K.M., Seinen, A.B., Shimizu, T.S., and Tans, S.J. (2020). Bacterial  
882 coexistence driven by motility and spatial competition. *Nature* *578*, 588-592.

- 
- 883 49. Paulose, J., and Hallatschek, O. (2020). The impact of long-range dispersal on gene  
884 surfing. *Proc. Natl. Acad. Sci. U. S. A.* *117*, 7584-7593.
- 885 50. Lilja, E.E., and Johnson, D.R. (2016). Segregating metabolic processes into different  
886 microbial cells accelerates the consumption of inhibitory substrates. *ISME J.* *10*, 1568-  
887 1578.
- 888 51. Silvertown, J., Dodd, M.E., Gowing, D.J.G., and Mountford, J.O. (1999). Hydrologically  
889 defined niches reveal a basis for species richness in plant communities. *Nature* *400*, 61-  
890 63.
- 891 52. Hart, S.P., Usinowicz, J., and Levine, J.M. (2017). The spatial scales of species  
892 coexistence. *Nat. Ecol. Evol.* *1*, 1066-1073.
- 893 53. Weinstein, B.T., Lavrentovich, M.O., Mobius, W., Murray, A.W., and Nelson, D.R. (2017).  
894 Genetic drift and selection in many-allele range expansions. *PLOS Comput. Biol.* *13*,  
895 e1005866.
- 896 54. Davies, S., White, A., and Lowe, A. (2004). An investigation into effects of long-distance  
897 seed dispersal on organelle population genetic structure and colonization rate: A model  
898 analysis. *Heredity* *93*, 566-576.
- 899 55. Bialozyt, R., Ziegenhagen, B., and Petit, R.J. (2006). Contrasting effects of long distance  
900 seed dispersal on genetic diversity during range expansion. *J. Evol. Biol.* *19*, 12-20.
- 901 56. Fayard, J., Klein, E.K., and Lefevre, F. (2009). Long distance dispersal and the fate of a  
902 gene from the colonization front. *J. Evol. Biol.* *22*, 2171-2182.
- 903 57. Haq, I.U., Calixto, R.O.D., Yang, P., dos Santos, G.M.P., Barreto-Bergter, E., and van Elsas,  
904 J.D. (2016). Chemotaxis and adherence to fungal surfaces are key components of the  
905 behavioral response of *Burkholderia terrae* BS001 to two selected soil fungi. *FEMS*  
906 *Microbiol. Ecol.* *92*, fiw164.

- 
- 907 58. Frey-Klett, P., Burlinson, P., Deveau, A., Barret, M., Tarkka, M., and Sarniguet, A. (2011).  
908 Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and  
909 food microbiologists. *Microbiol. Mol. Biol. Rev.* 75, 583-609.
- 910 59. Chesson, P. (2000). General theory of competitive coexistence in spatially-varying  
911 environments. *Theor. Popul. Biol.* 58, 211-237.
- 912 60. Dominguez, J., Aira, M., Crandall, K.A., and Pérez-Losada, M. (2021). Earthworms  
913 drastically change fungal and bacterial communities during vermicomposting of sewage  
914 sludge. *Sci. Rep.* 11, 15556.
- 915 61. Ruddick, S.M., and Williams, S.T. (1972). Studies on the ecology of actinomycetes in soil  
916 V. Some factors influencing the dispersal and adsorption of spores in soil. *Soil Biol.*  
917 *Biochem.* 4, 93-100.
- 918 62. Trevors, J.T., van Elsas, J.D., van Overbeek, L.S., and Starodub, M.E. (1990). Transport of  
919 ggeenetically engineered *Pseudomonas fluorescens* strain through a soil microcosm.  
920 *Appl. Environ. Microbiol.* 56, 401-408.
- 921 63. Tecon, R., Ebrahimi, A., Kleyer, H., Levi, S.E., and Or, D. (2018). Cell-to-cell bacterial  
922 interactions promoted by drier conditions on soil surfaces. *Proc. Natl. Acad. Sci. U. S. A.*  
923 115, 9791-9796.
- 924 64. Ruan, C., Ramoneda, J., Chen, G., Johnson, D.R., and Wang, G. (2021). Evaporation-  
925 induced hydrodynamics promote conjugation-mediated plasmid transfer in microbial  
926 populations. *ISME Comm.* 1, 54.
- 927 65. Daane, L.L., Molina, J.A., Berry, E.C., and Sadowsky, M.J. (1996). Influence of earthworm  
928 activity on gene transfer from *Pseudomonas fluorescens* to indigenous soil bacteria.  
929 *Appl. Environ. Microbiol.* 62, 515-521.

- 
- 930 66. Lilja, E.E., and Johnson, D.R. (2019). Substrate cross-feeding affects the speed and  
931 trajectory of molecular evolution within a synthetic microbial assemblage. *BMC Evol.*  
932 *Biol.* *19*, 129.
- 933 67. Wang, S., Yu, S., Zhang, Z., Wei, Q., Yan, L., Ai, G., Liu, H., and Ma, L.Z. (2014).  
934 Coordination of swarming motility, biosurfactant synthesis, and biofilm matrix  
935 exopolysaccharide production in *Pseudomonas aeruginosa*. *Appl. Environ. Microbiol.* *80*,  
936 6724-6732.
- 937 68. Wang, S.W., Parsek, M.R., Wozniak, D.J., and Ma, L.Y.Z. (2013). A spider web strategy of  
938 type IV pili-mediated migration to build a fibre-like Psl polysaccharide matrix in  
939 *Pseudomonas aeruginosa* biofilms. *Environ. Microbiol.* *15*, 2238-2253.
- 940 69. Nilsson, R.H., Taylor, A.F.S., Adams, R.I., Baschien, C., Bengtsson-Palme, J., Cangren, P.,  
941 Coleine, C., Daniel, H.M., Glassman, S.I., Hirooka, Y., et al. (2018). Taxonomic annotation  
942 of public fungal ITS sequences from the built environment - a report from an April 10-11,  
943 2017 workshop (Aberdeen, UK). *Myckeys* *28*, 65-82.
- 944 70. R Core Team. (2016). R: A language and environment for statistical computing (R Core  
945 Team, Vienna, Austria).
- 946 71. Meier, P., Berndt, C., Weger, N., and Wackernagel, W. (2002). Natural transformation of  
947 *Pseudomonas stutzeri* by single-stranded DNA requires type IV pili, competence state  
948 and *comA*. *FEMS Microbiol. Lett.* *207*, 75-80.
- 949 72. Gardes, M., and Bruns, T.D. (1993). ITS primers with enhanced specificity for  
950 basidiomycetes - application to the identification of mycorrhizae and rusts. *Mol. Ecol.* *2*,  
951 113-118.
- 952 73. Ciccarese, D., Micali, G., Borer, B., Ruan, C., Or, D., and Johnson, D.R. (2022). Rare and  
953 localized events stabilize microbial community composition and patterns of spatial self-  
954 organization in a fluctuating environment. *ISME J.* *16*, 1453-1463.

- 
- 955 74. Prest, E.I., Hammes, F., Kötzsch, S., van Loosdrecht, M.C.M., and Vrouwenvelder, J.S.  
956 (2013). Monitoring microbiological changes in drinking water systems using a fast and  
957 reproducible flow cytometric method. *Water Res.* 47, 7131-7142.
- 958 75. Reisner, A., Molin, S., and Zechner, E.L. (2002). Recombinogenic engineering of  
959 conjugative plasmids with fluorescent marker cassettes. *FEMS Microbiol. Ecol.* 42, 251-  
960 259.

## KEY RESOURCES TABLE

| REAGENT or RESOURCE                                 | SOURCE   | IDENTIFIER  |
|---|--|---|
| <b>Chemicals</b>                                    |  |   |
| Sodium nitrate                                      | Sigma-Aldrich  | S5506   |
| Isopropyl $\beta$ -D-1-thiogalactopyranoside        | Sigma-Aldrich  | I6758   |
| Lysogeny broth                                      | Sigma-Aldrich  | L1900   |
| Lysogeny broth agar                                 | Sigma-Aldrich  | L2025   |
| Kanamycin disulfate                                 | Sigma-Aldrich  | K1876   |
| <b>Experimental models:<br/>Organisms/strains</b>   |  |   |
| <i>P. stutzeri</i> A1601- <i>egfp</i>               | Lilja and Johnson <sup>50</sup> ,<br>Lilja and Johnson <sup>66</sup> | N/A   |
| <i>P. stutzeri</i> A1601- <i>ech</i>                | Lilja and Johnson <sup>50</sup> ,<br>Lilja and Johnson <sup>66</sup> | N/A   |
| <i>P. stutzeri</i> A1601- <i>ecfp</i>               | Lilja and Johnson <sup>50</sup> ,<br>Lilja and Johnson <sup>66</sup> | N/A   |
| <i>P. stutzeri</i> A1602- <i>egfp</i>               | Lilja and Johnson <sup>50</sup> ,<br>Lilja and Johnson <sup>66</sup> | N/A   |
| <i>P. stutzeri</i> A1603- <i>ecfp</i>               | Lilja and Johnson <sup>50</sup> ,<br>Lilja and Johnson <sup>66</sup> | N/A   |
| <i>P. aeruginosa</i> PAO1- <i>gfp</i>               | Wang et al. <sup>67</sup>  | N/A   |
| <i>P. aeruginosa</i> PAO1- <i>rfp</i>               | Wang et al. <sup>68</sup>  | N/A   |
| <i>P. aeruginosa</i> PAO1- $\Delta$ <i>fliC-rfp</i> | Wang et al. <sup>67</sup> , Wang<br>et al. <sup>68</sup>             | N/A   |
| <i>P. aeruginosa</i> PAO1- $\Delta$ <i>fliC-gfp</i> | Wang et al. <sup>67</sup> , Wang<br>et al. <sup>68</sup>             | N/A   |
| <i>P. aeruginosa</i> PAO1- $\Delta$ <i>pilA-rfp</i> | Wang et al. <sup>67</sup> , Wang<br>et al. <sup>68</sup>             | N/A   |
| <i>Penicillium</i> sp. laika                        | This study   | N/A   |
| <b>Deposited data</b>                               |  |   |
| Original code                                       | ERIC open  | <a href="https://doi.org/10.25678/0007GJ">https://doi.org/10.25678/0007GJ</a>                   |
| Experimental data                                   | ERIC open  | <a href="https://doi.org/10.25678/0007GJ">https://doi.org/10.25678/0007GJ</a>                   |
| Sequence data                                       | Genbank  | MG818940.1, KT270333.1,<br>KM396384.1, KM396380.1   |
| <b>Software and algorithms</b>                      |  |   |
| UNITE   | Nilsson et al. <sup>69</sup>   | <a href="https://unite.ut.ee/">https://unite.ut.ee/</a>   |
| BLAST   | NCBI   | <a href="https://blast.ncbi.nlm.nih.gov/Blast.cgi">https://blast.ncbi.nlm.nih.gov/Blast.cgi</a> |
| Fiji (v1.53c)                                       | Fiji   | <a href="https://fiji.sc">https://fiji.sc</a>   |
| ImageJ  | ImageJ   | <a href="https://imagej.net">https://imagej.net</a>   |
| R/RStudio   | R Core Team <sup>70</sup>  | <a href="http://www.Rproject.org/">http://www.Rproject.org/</a>                                 |

Figure 1

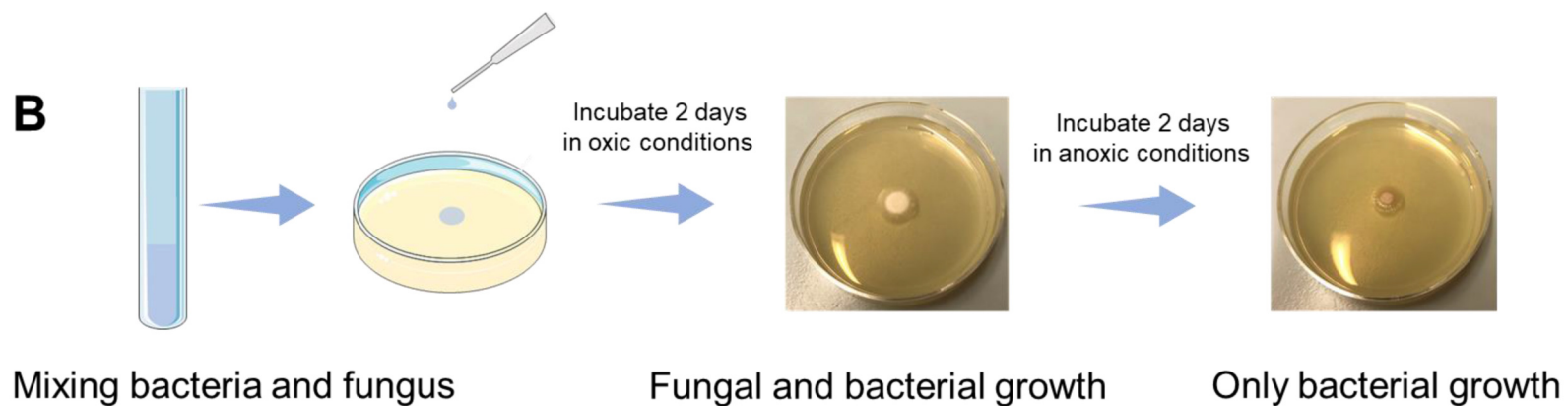
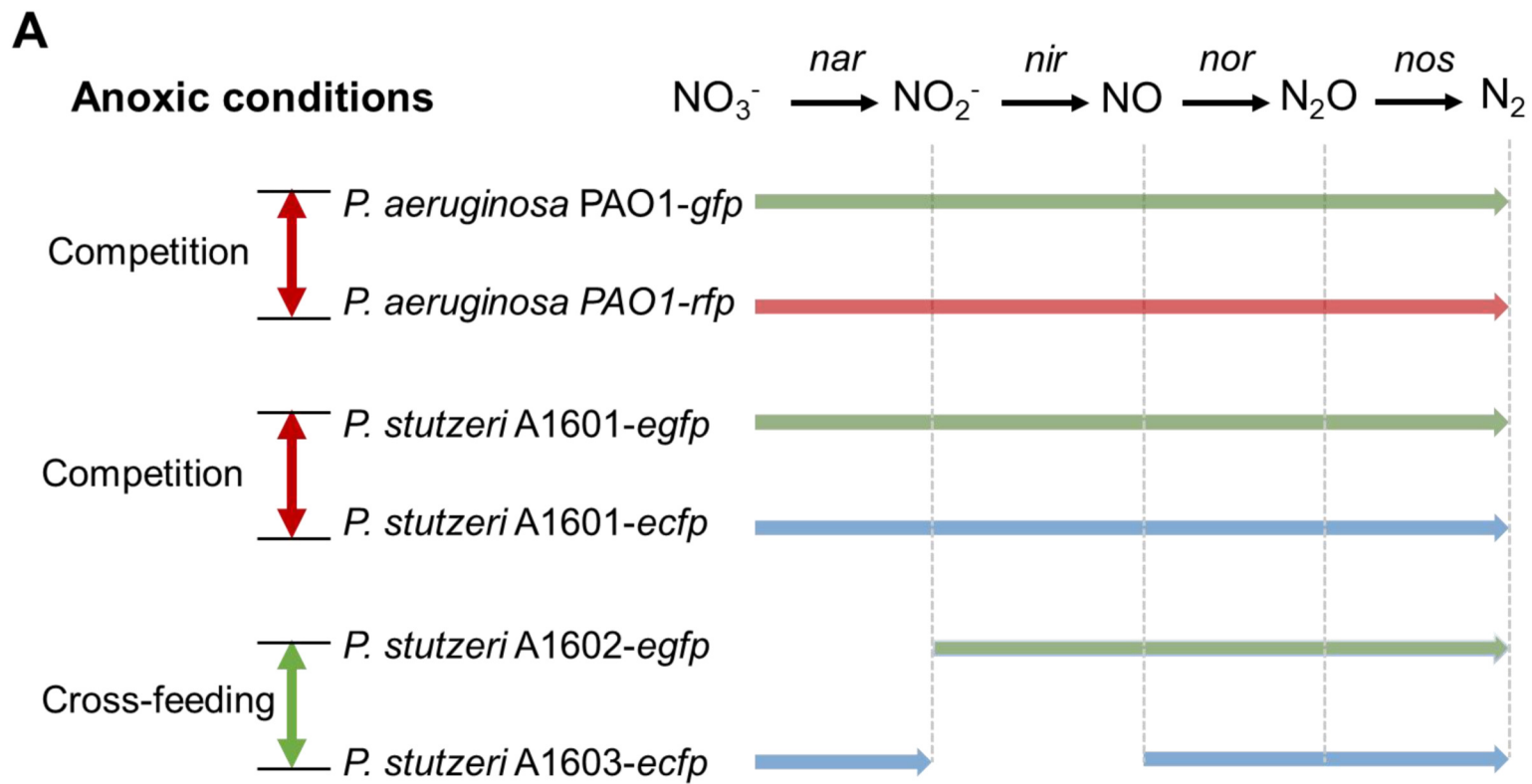


Figure 2

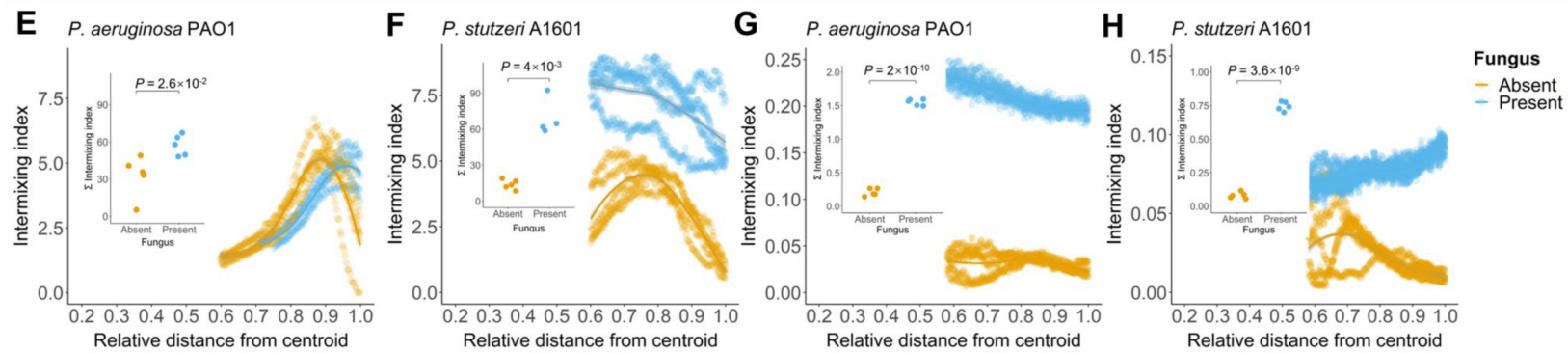
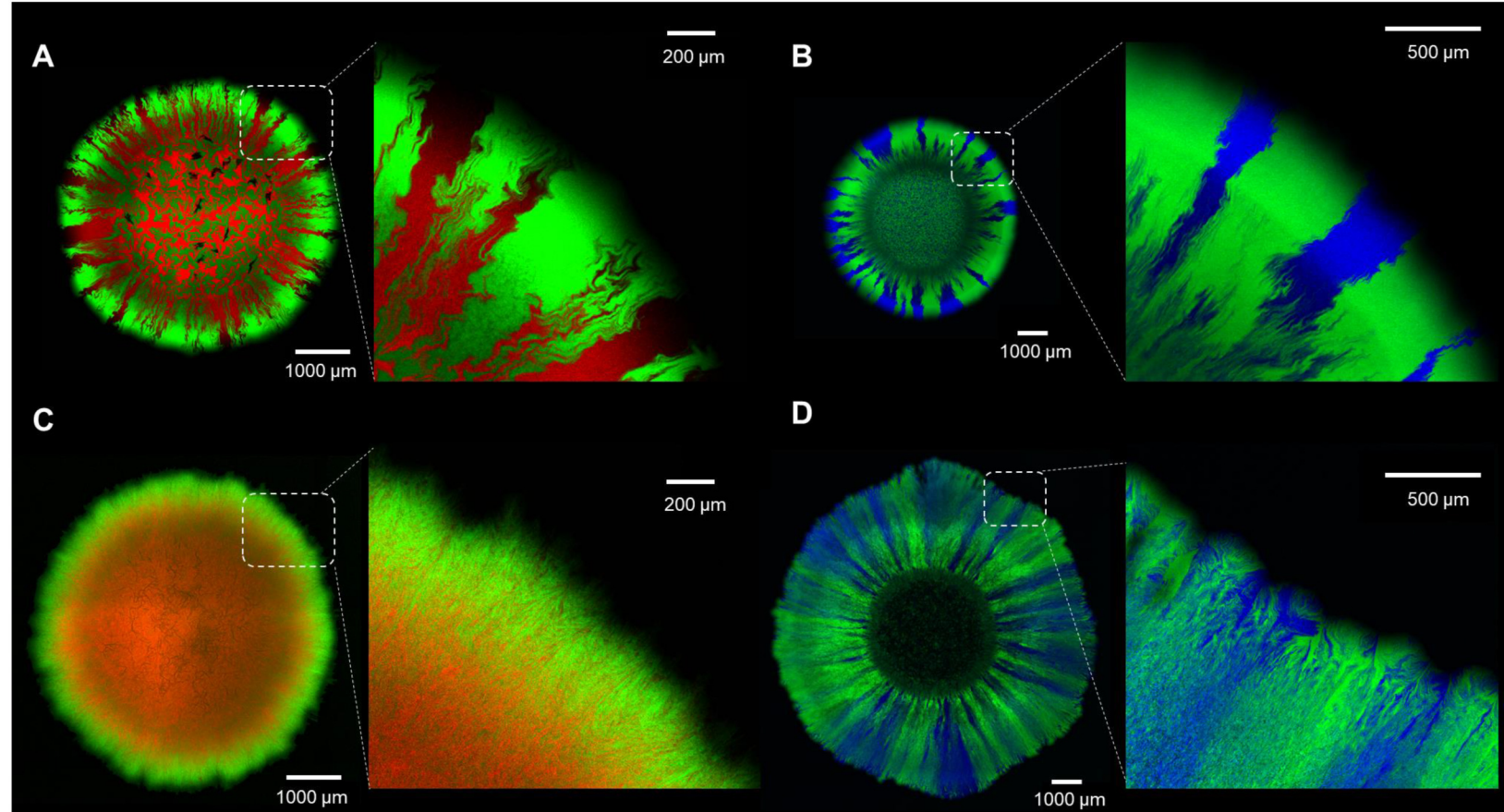




Figure 3

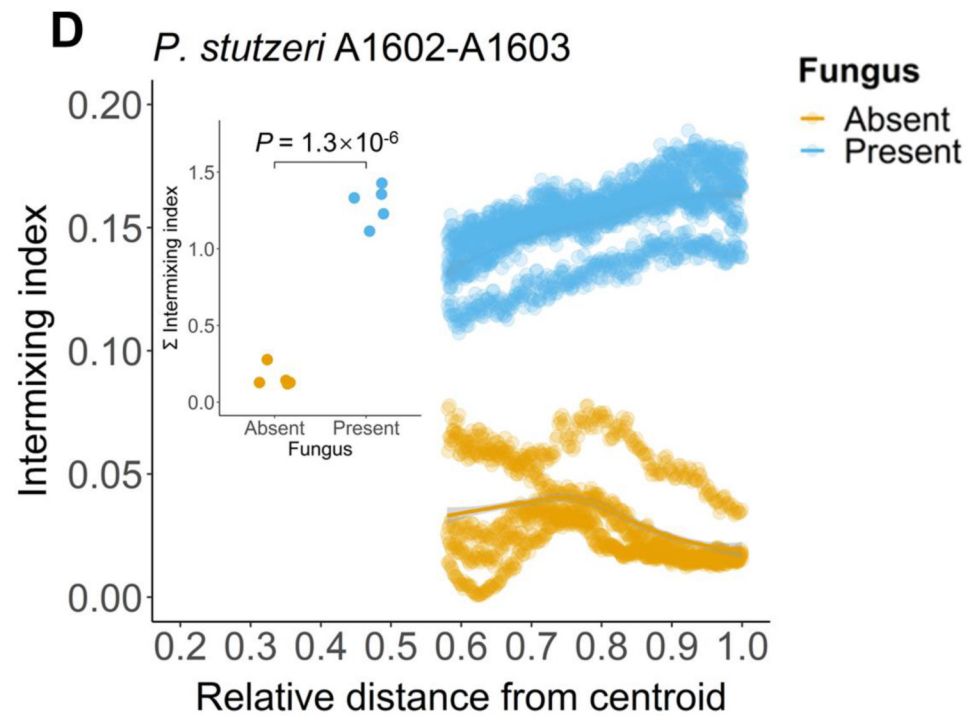
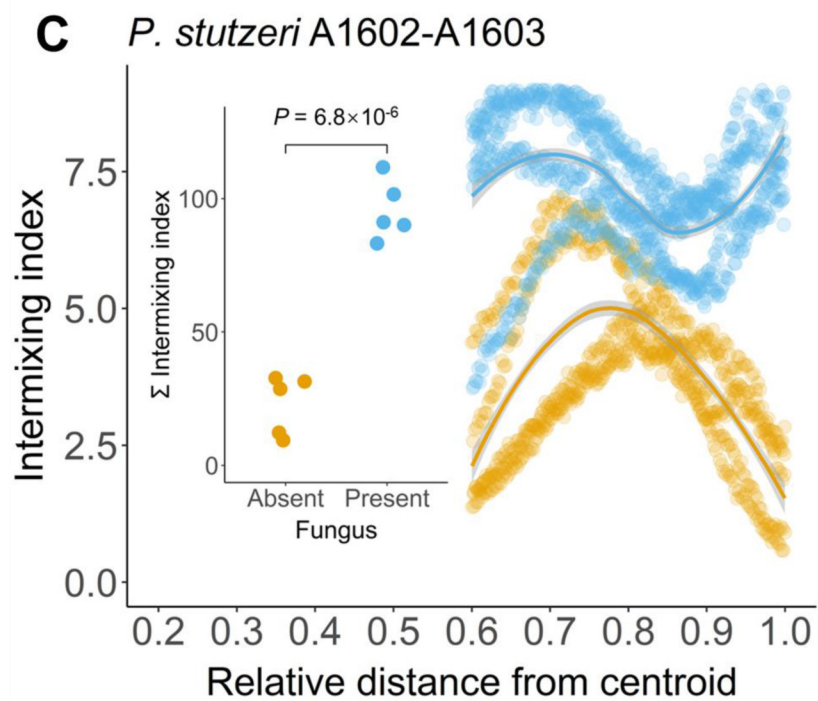
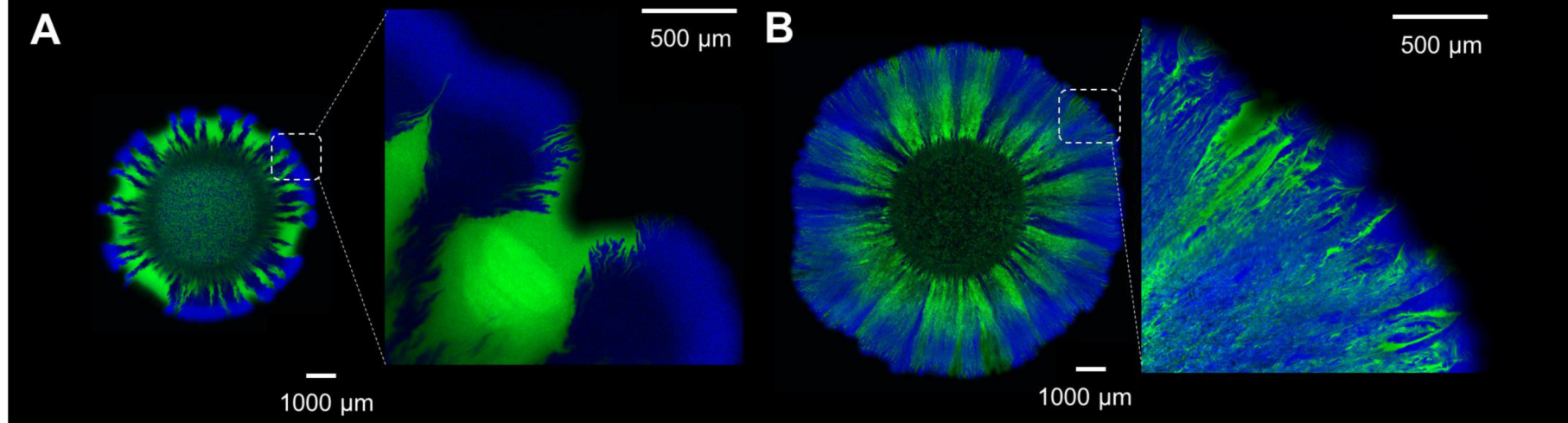
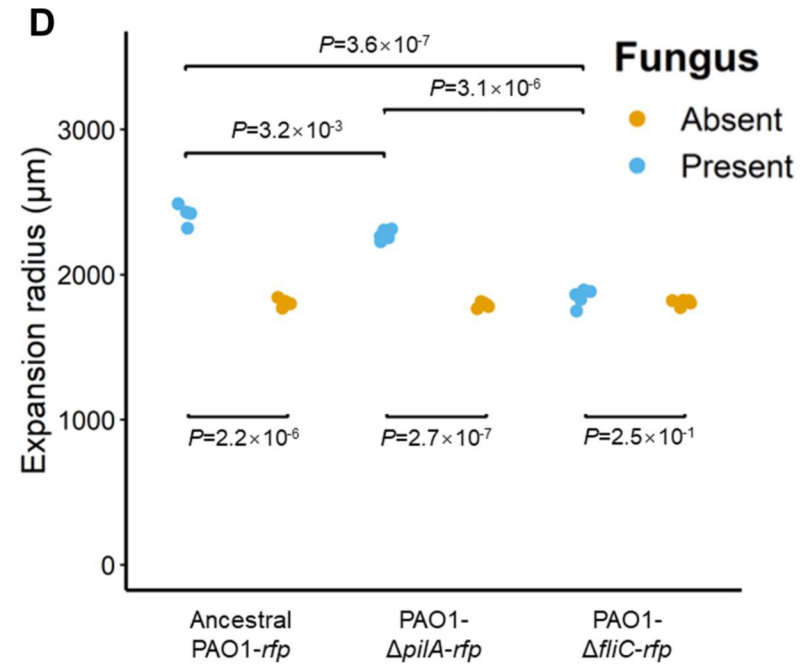
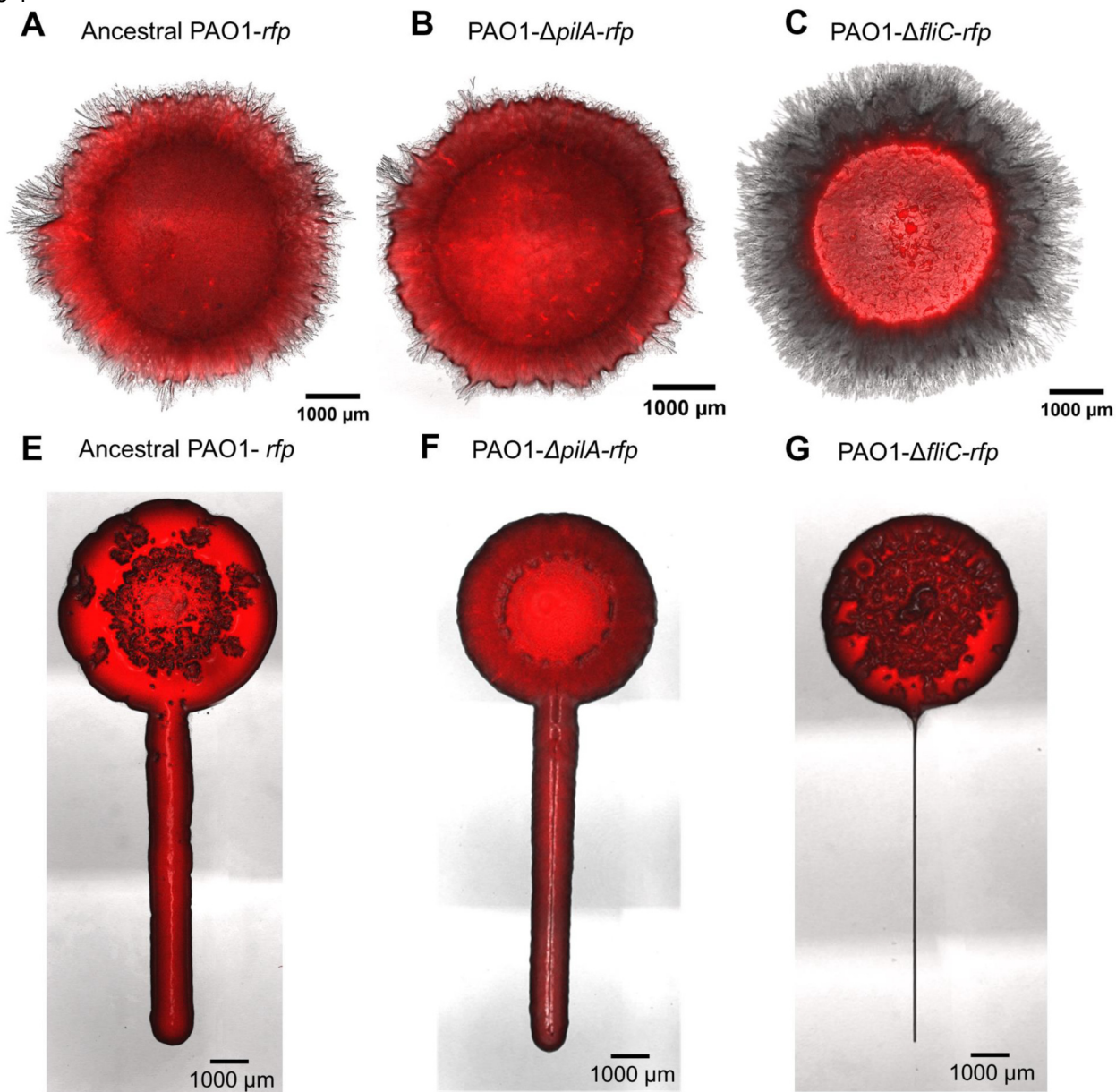


Figure 4



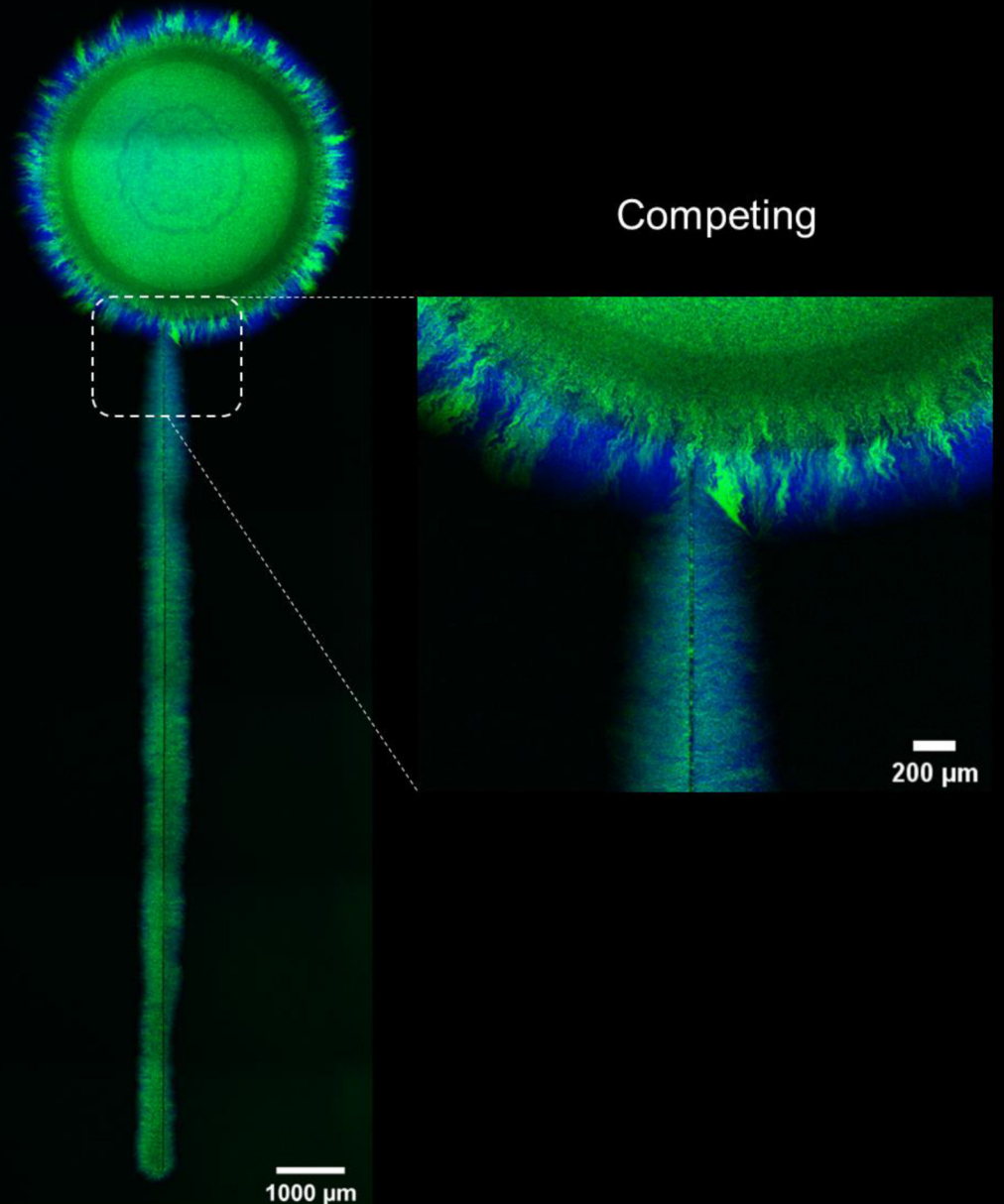
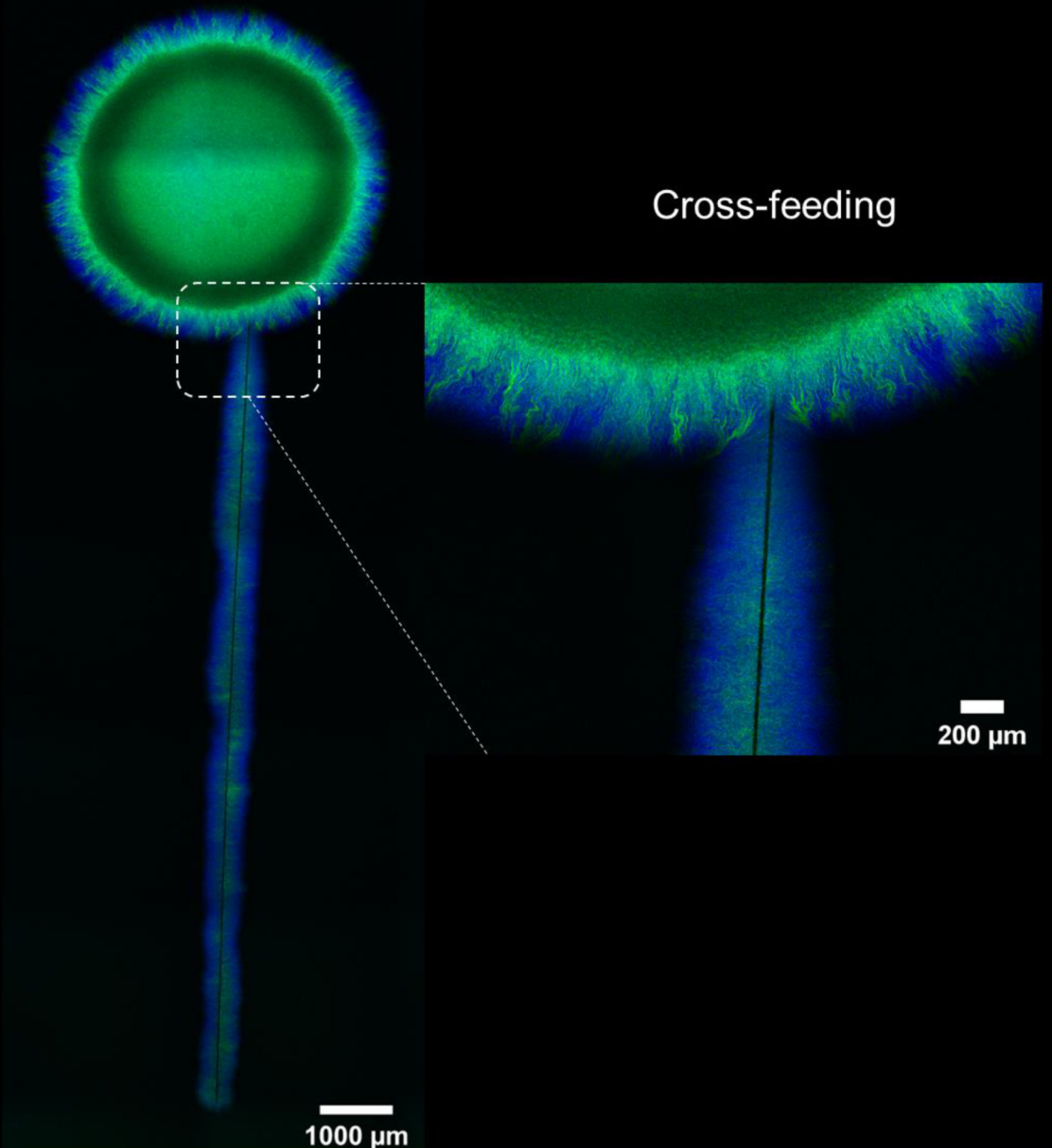
**A****B**



Figure 6

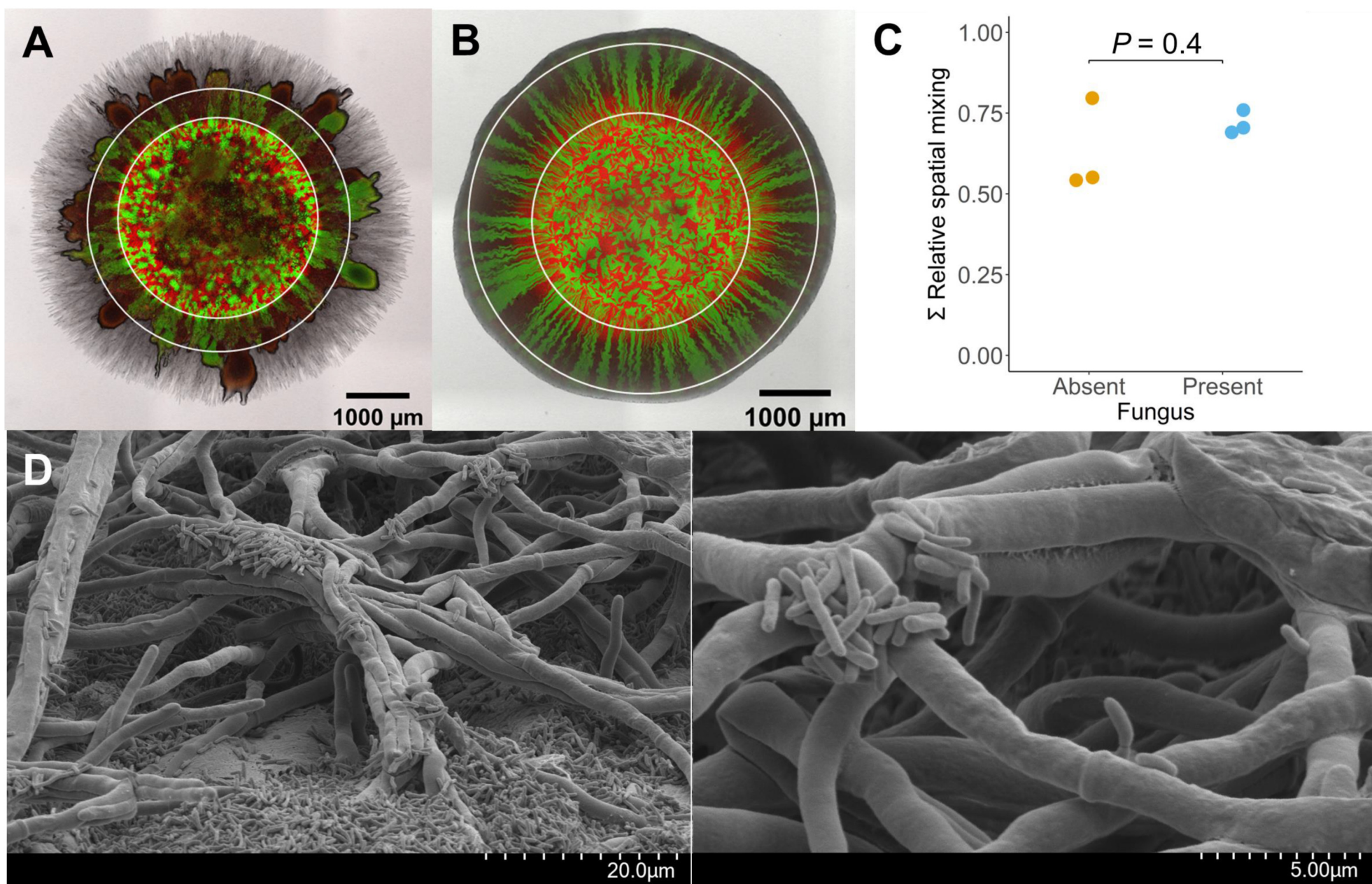
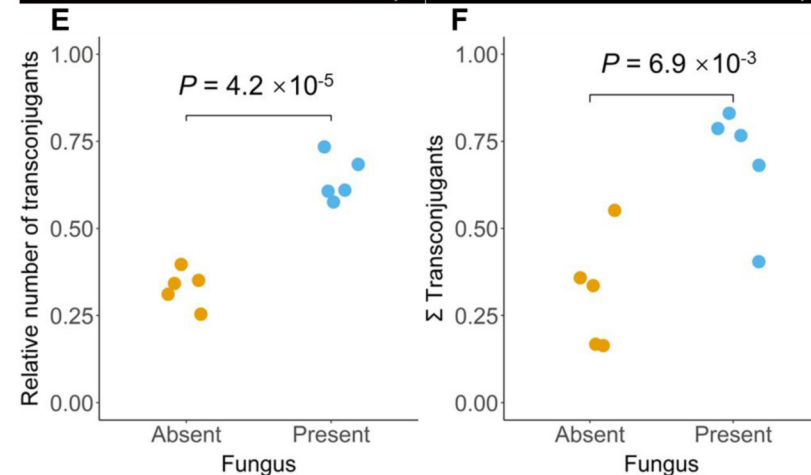
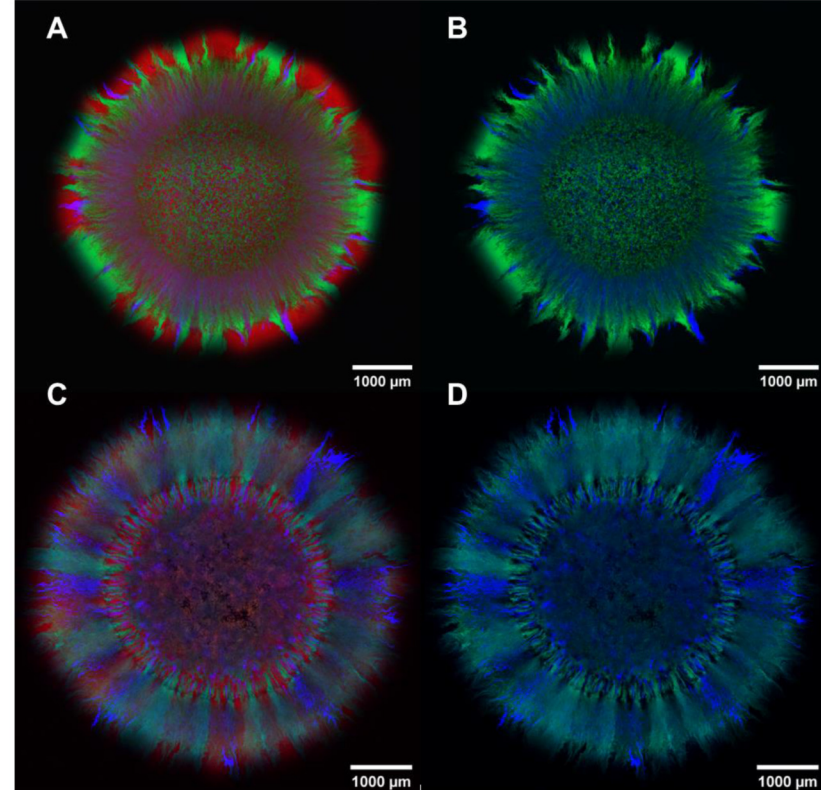
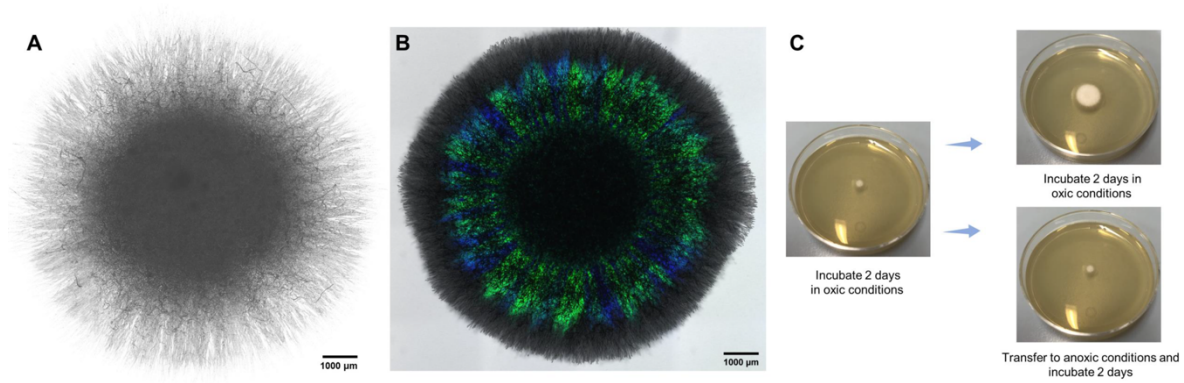
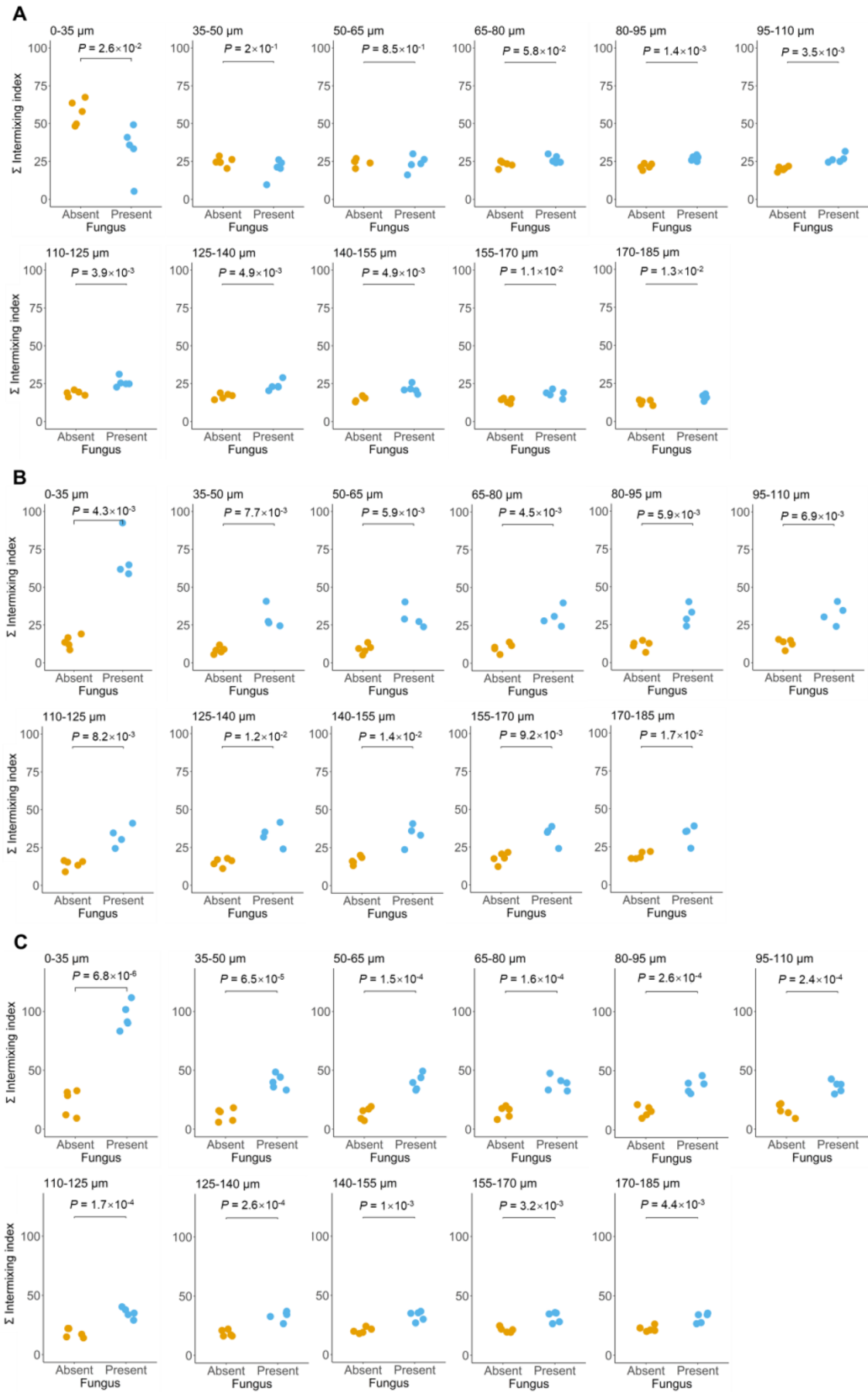


Figure 7





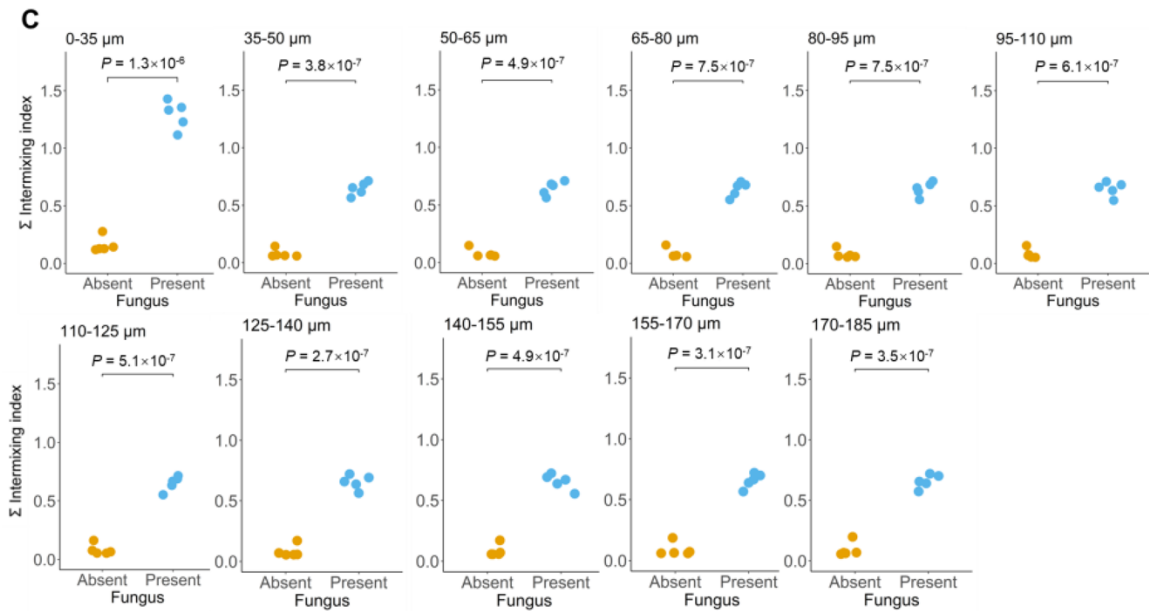
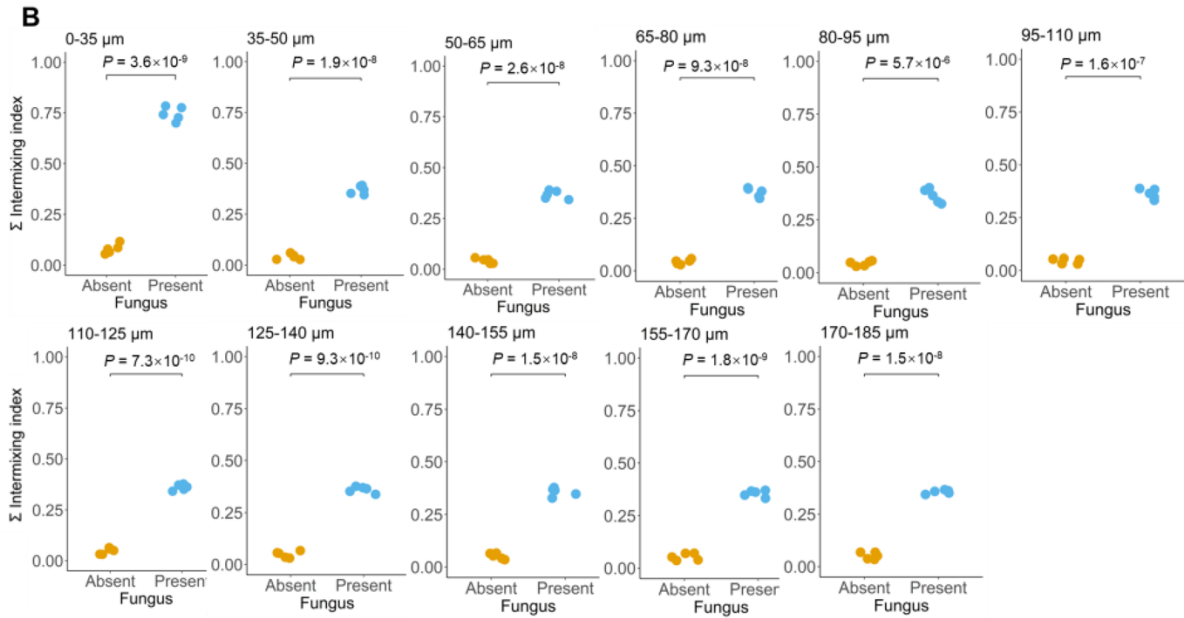
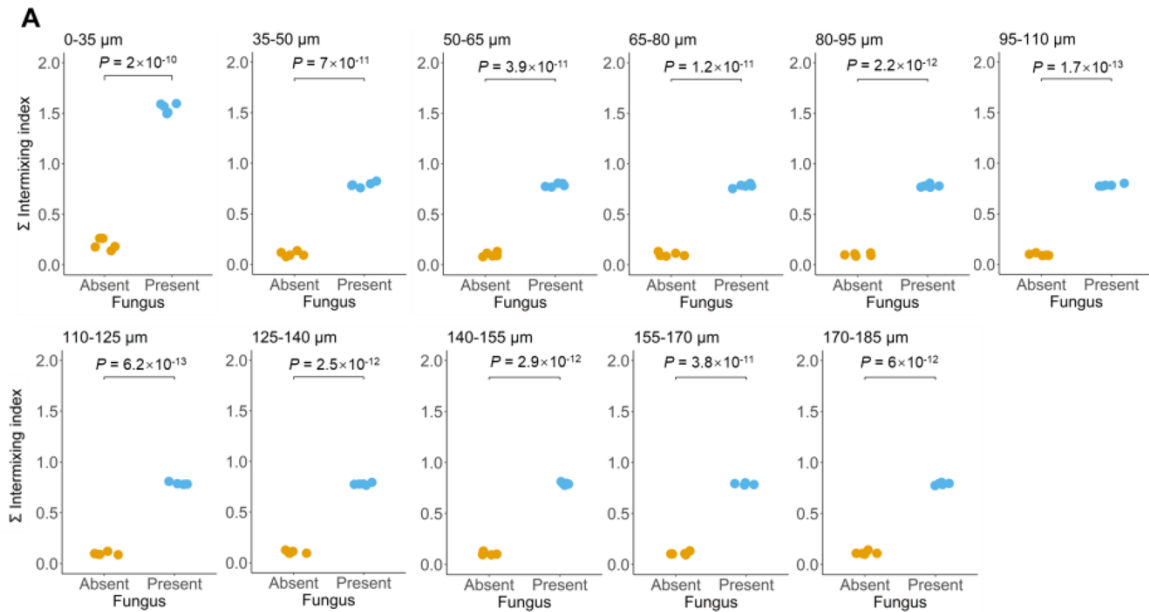
**Figure S1. Hyphal network formed by *Penicillium sp. laika* and comparison of its growth in oxic and anoxic conditions, related to Figure 1. A,** Representative confocal laser scanning microscopy image of the hyphal network. We inoculated *Penicillium sp. laika* alone and grew it on nutrient-amended agar plates for two days in continuous oxic conditions. **B,** Representative confocal laser scanning microscopy image for the competing pair of *P. stutzeri* A1601 strains in the presence of *Penicillium sp. laika*. We inoculated the bacterial and fungal strains together and grew them on nutrient-amended agar plates for two days in continuous oxic conditions. **C,** Growth of *Penicillium sp. laika* was immediately inhibited when we transferred the agar plates to anoxic conditions.



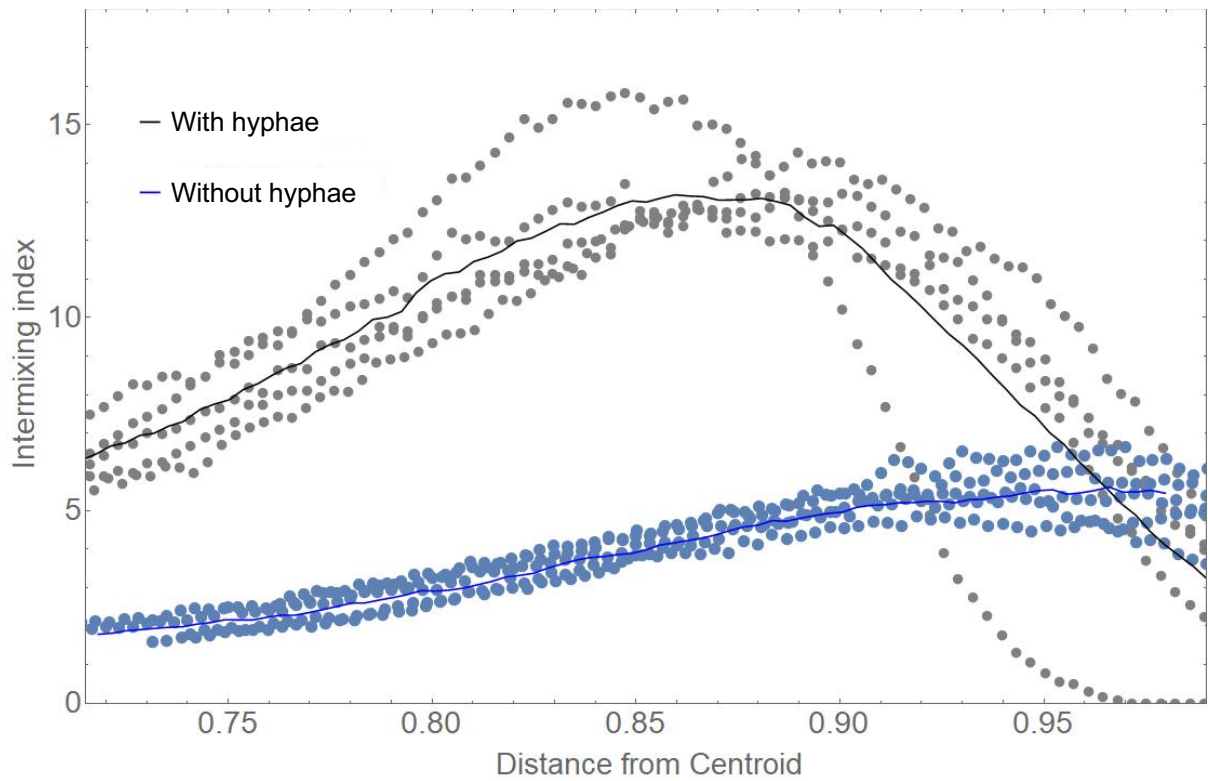


**Figure S2. Quantification of the intermixing index using the local scale Fourier transformation method at 15  $\mu\text{m}$  increments behind the expansion frontier after range expansion, related to Figures 2 and 3.** We mixed pairs of the competing *P. aeruginosa* PAO1 strains, the competing *P. stutzeri* A1601 strains, or the cross-feeding *P. stutzeri* A1602 and A1603 strains together and allowed them to expand across a nutrient-amended agar surface in the absence or presence of *Penicillium* sp. laika for four days as described in Figure 1b. At the end of the range expansion experiment, we quantified the intermixing index across the expansion region using the local scale Fourier transform method. Quantities are for pairs of **A** competing *P. aeruginosa* PAO1 strains, **B** competing *P. stutzeri* A1601 strains, or **C** cross-feeding *P. stutzeri* A1602 and A1603 strains. Each data point is the measurement for an independent experimental replicate ( $n = 5$ ) and *P* is for a two-sample two-sided Welch test with a Holm-Bonferroni correction.

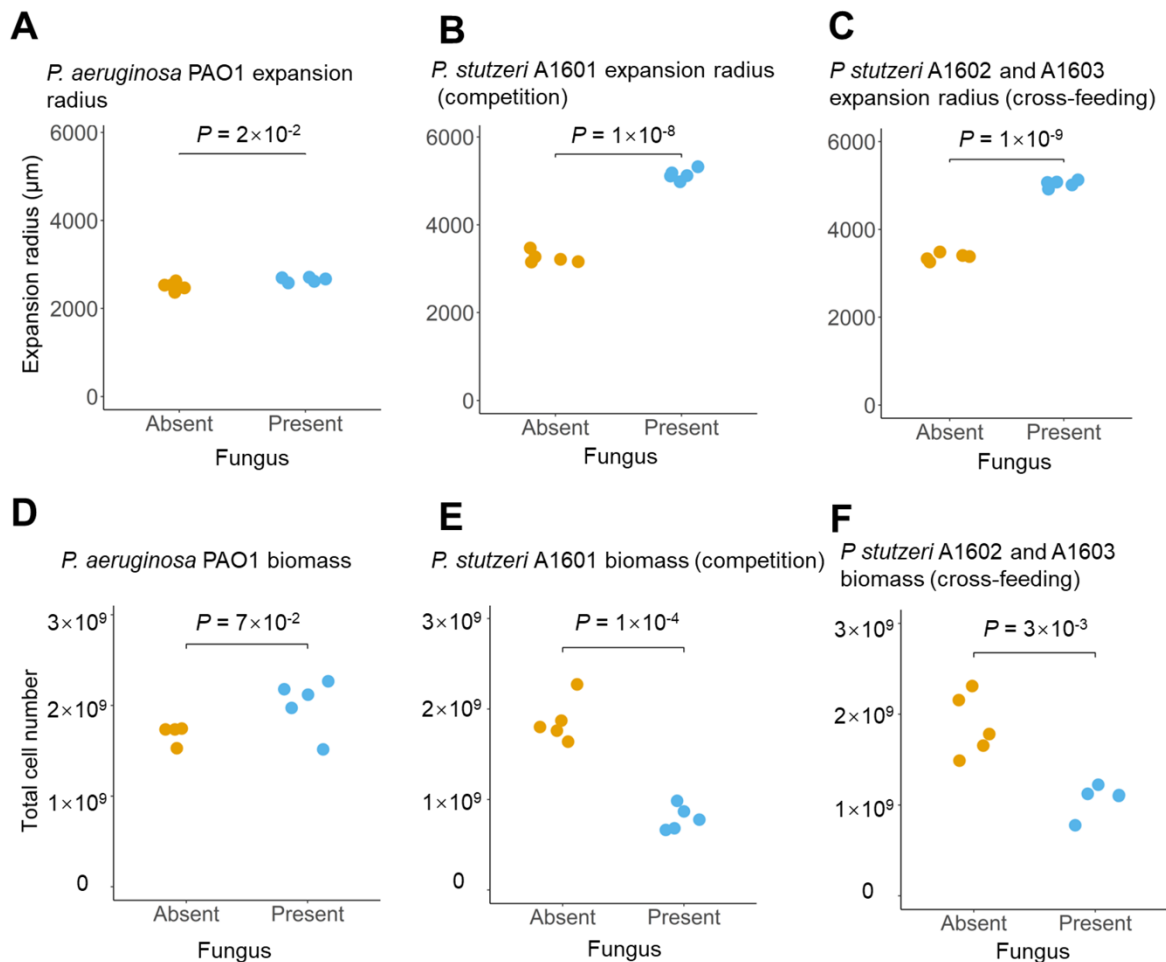




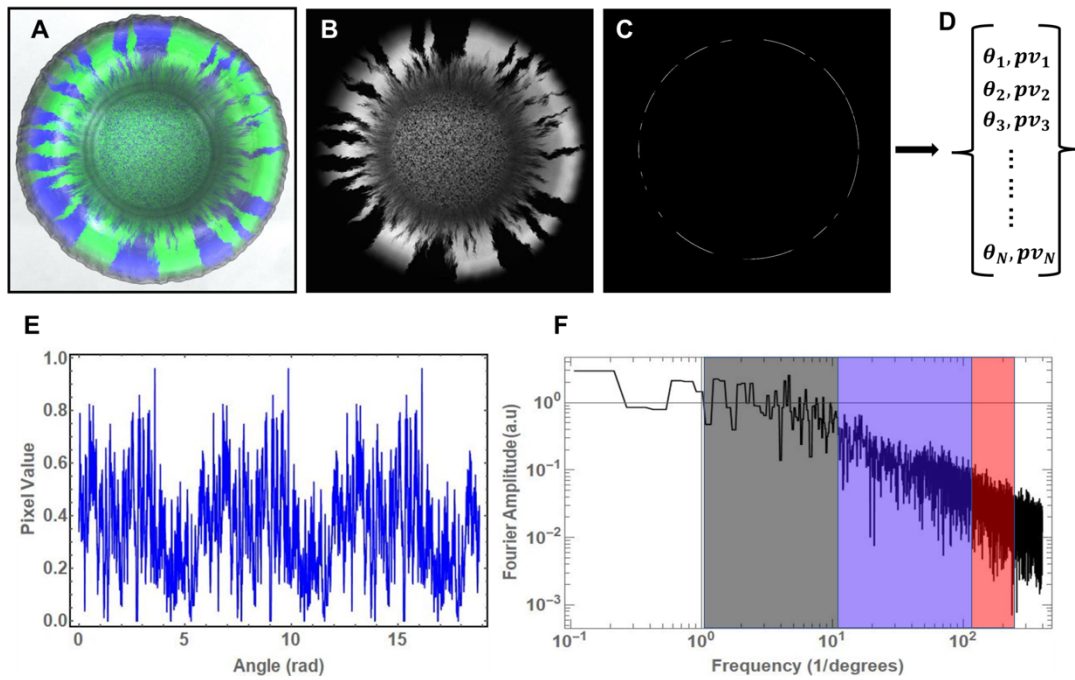
**Figure S3. Quantification of the intermixing index using the intersection method at 15  $\mu\text{m}$  increments behind the expansion frontier after range expansion, related to Figures 2 and 3.** We mixed pairs of the competing *P. aeruginosa* PAO1 strains, the competing *P. stutzeri* A1601 strains, or the cross-feeding *P. stutzeri* A1602 and A1603 strains together and allowed them to expand across a nutrient-amended agar surface in the absence or presence of *Penicillium* sp. laika for four days as described in Figure 1b. At the end of the range expansion experiment, we quantified the intermixing index across the expansion region using the intersection method. Quantities are for pairs of **A** competing *P. aeruginosa* PAO1 strains, **B** competing *P. stutzeri* A1601 strains, or **C** cross-feeding *P. stutzeri* A1602 and A1603 strains. Each data point is the measurement for an independent experimental replicate ( $n = 5$ ) and  $P$  is for a two-sample two-sided Welch test with a Holm-Bonferroni correction.



**Figure S4. Intermixing index quantified at the intermediate length scale for range expansions of the competing pair of *P. aeruginosa* PAO1 strains in the absence or presence of fungal hyphae, related to Figure 2.** We quantified the intermixing at the intermediate length scale from the images presented in Figure 2A and 2C. We quantified the intermixing index in the radial direction from the outer edge of the inoculation area to the outer edge of the final expansion frontier at the end of the range expansion experiment. The lines are the moving averages of the intermixing index. Each data point is the measurement for an independent experimental replicate (n = 5).



**Figure S5. Expansion area and total biomass of bacterial range expansions in the absence or presence of fungal hyphae, related to Figures 2 and 3.** Radii of expansion areas for **A** the competing pair of *P. aeruginosa* PAO1 strains, **B** the competing pair of *P. stutzeri* A1601 strains, or **C** the cross-feeding pair of *P. stutzeri* A1602 and A1603 strains after four days of range expansion across a nutrient-amended agar surface in the absence or presence of *Penicillium* sp. laika. Total cell numbers within the expansion areas for **D** the competing pair of *P. aeruginosa* PAO1 strains, **E** the competing pair of *P. stutzeri* A1601 strains, or **F** the cross-feeding pair of *P. stutzeri* A1602 and A1603 strains after four days of range expansion across a nutrient-amended agar surface in the absence or presence of *Penicillium* sp. laika. We measured the expansion radii and quantified the total cell numbers from the same experiments as described in the main text. Each data point is the measurement for an independent experimental replicate ( $n = 5$ ) and  $P$  is for a two-sample two-sided Welch test.



**Figure S6. Image analysis and quantification of spatial patterns using Fourier transforms, related to STAR Methods.** **A**, CLSM image of a range expansion with competing strains of *P. stutzeri* A1601-*egfp* and A1601-*ecfp* (green and blue) in the absence of *Penicillium* sp. laika. **B**, Grayscale image of the green layer from **A**. **C**, From **B**, we obtained a 1-pixel-wide ring at a radius of 700 pixels and enhanced the contrast of the image for improved visualization. **D**, We transformed **C** into a series of angles and pixel values, where we calculated the angles in radians from the positive x-direction. **E**, We copied the data from **D** twice and appended the data to original time-series. This step is necessary to account for the circular periodicity of the data. **F**, We performed Fourier transforms of the data in **E**. The different shades correspond to different scales of mixing. Dark grey corresponds to small frequencies, which is equivalent to periodicity on the scale of 5 to 50 degrees (global scales). Blue corresponds to immediate frequencies, which is equivalent to periodicity on the scale of 0.5 to 5 degrees (intermediate scales). Red corresponds to large frequencies, which is equivalent to periodicity on the scale of 0.2 to 0.5 degrees (local scales).

| Strain  | Relevant characteristics  | Reference  |
|---|---|------------|
| <i>P. stutzeri</i> A1601- <i>egfp</i>                 | A1501 with $\Delta comA$ and mini-Tn7T-LAC-Gm- <i>egfp</i> ; Gm <sup>R</sup> , <i>egfp</i> <sup>+</sup>   | S1, S2     |
| <i>P. stutzeri</i> A1601- <i>ech</i>                  | A1501 with $\Delta comA$ and mini-Tn7T-LAC-Gm- <i>ech</i> ; Gm <sup>R</sup> , <i>ech</i> <sup>+</sup>   | S1, S2     |
| <i>P. stutzeri</i> A1601- <i>ecfp</i>                 | A1501 with $\Delta comA$ and mini-Tn7T-LAC-Gm- <i>ecfp</i> ; Gm <sup>R</sup> , <i>ecfp</i> <sup>+</sup>   | S1, S2     |
| <i>P. stutzeri</i> A1602- <i>egfp</i>                 | A1502 with $\Delta comA$ , $\Delta narG$ and mini-Tn7T-LAC-Gm- <i>egfp</i> ; Gm <sup>R</sup> , <i>egfp</i> <sup>+</sup>                                       | S1, S2     |
| <i>P. stutzeri</i> A1603- <i>ecfp</i>                 | A1503 with $\Delta comA$ , $\Delta nirS$ and mini-Tn7T-LAC-Gm- <i>ecfp</i> ; Gm <sup>R</sup> , <i>ecfp</i> <sup>+</sup>                                       | S1, S2     |
| <i>P. aeruginosa</i> PAO1- <i>gfp</i>                 | PAO1 with plasmid pSMC21::Kam <sup>R</sup> - <i>gfp</i>   | S3         |
| <i>P. aeruginosa</i> PAO1- <i>rfp</i>                 | PAO1 with plasmid pBRM::Kam <sup>R</sup> - <i>rfp</i>   | S4         |
| <i>P. aeruginosa</i> PAO1- $\Delta fliC$ - <i>rfp</i> | PAO1 with $\Delta fliC$ in-frame deletion; plasmid pBRM::Kam <sup>R</sup> - <i>rfp</i>  | S3, S4     |
| <i>P. aeruginosa</i> PAO1- $\Delta fliC$ - <i>gfp</i> | PAO1 with $\Delta fliC$ in-frame deletion; plasmid pSMC21::Kam <sup>R</sup> - <i>gfp</i>  | S3, S4     |
| <i>P. aeruginosa</i> PAO1- $\Delta pilA$ - <i>rfp</i> | PAO1 with $\Delta pilA$ in-frame deletion; plasmid pBRM::Kam <sup>R</sup> - <i>rfp</i>  | S3, S4     |
| <i>Penicillium</i> sp. laika                          | Colony morphology on agar: Filamentous, raised, rough surface, filiform margin, and white color after growth on lysogeny broth agar medium at 20°C for 4 days | This study |
| plasmid pAR145ecfp                                    | pSU2007 <i>aph</i> :: <i>cat</i> -PA1/04/03- <i>cfp</i> -T0   | S5         |

**Table S1. Specifications of the strains and plasmid used in this study, related to STAR Methods.**

## Supplemental Reference List

- S1 Lilja, E.E., and Johnson, D.R. (2016). Segregating metabolic processes into different microbial cells accelerates the consumption of inhibitory substrates. *ISME J.* *10*, 1568-1578.
- S2 Lilja, E.E., and Johnson, D.R. (2019). Substrate cross-feeding affects the speed and trajectory of molecular evolution within a synthetic microbial assemblage. *BMC Evol. Biol.* *19*, 129.
- S3 Wang, S., Yu, S., Zhang, Z., Wei, Q., Yan, L., Ai, G., Liu, H., and Ma, L.Z. (2014). Coordination of swarming motility, biosurfactant synthesis, and biofilm matrix exopolysaccharide production in *Pseudomonas aeruginosa*. *Appl. Environ. Microbiol.* *80*, 6724-6732.
- S4 Wang, S.W., Parsek, M.R., Wozniak, D.J., and Ma, L.Y.Z. (2013). A spider web strategy of type IV pili-mediated migration to build a fibre-like Psl polysaccharide matrix in *Pseudomonas aeruginosa* biofilms. *Environ. Microbiol.* *15*, 2238-2253.
- S5 Reisner, A., Molin, S., and Zechner, E.L. (2002). Recombinogenic engineering of conjugative plasmids with fluorescent marker cassettes. *FEMS Microbiol. Ecol.* *42*, 251-259.