Spatial scales of competition and a growth-motility tradeoff interact to determine bacterial coexistence

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Abstract
The coexistence of competing species is a long-lasting puzzle in evolutionary ecology research. Despite abundant experimental evidence showing that the opportunity for coexistence decreases as niche overlap increases between species, bacterial species and strains competing for the same resources are commonly found across diverse spatially heterogeneous habitats. We thus hypothesized that the spatial scale of competition may play a key role in determining bacterial coexistence, and interact with other mechanisms that promote coexistence, including a growth-motility tradeoff. To test this hypothesis, we let two Pseudomonas putida strains compete at local and regional scales by inoculating them either in a mixed droplet or in separate droplets in the same Petri dish, respectively. We also created conditions that allow the bacterial strains to disperse across abiotic or fungal hyphae networks. We found that competition at the local scale led to competitive exclusion while regional competition promoted coexistence. When competing in the presence of dispersal networks, the growth-motility tradeoff promoted coexistence only when the strains were inoculated in separate droplets. Our results provide a mechanism by which existing laboratory data suggesting competitive exclusion at a local scale is reconciled with the widespread coexistence of competing bacterial strains in complex natural environments with dispersal.

Introduction

Bacteria are important components of the microbial community in diverse habitats, and the coexistence of different bacterial species and strains can serve important ecosystem functions, from soil nutrient cycling to providing defense against pathogens for their animal and plant hosts [1–5]. Competition experiments under controlled laboratory conditions revealed a general trend that the closer the phylogenetic distance between the competing species, the more likely that one species/strain competitively excludes the other, since niche overlap and antagonistic interactions are generally more intense between closely related species [6–8]. Nevertheless, competing species with overlapping niche use are commonly observed in natural habitats, including soils [9,10], fermented foods [11,12], and different parts of the human body [13–16]. Various mechanisms have been identified to promote the coexistence of bacteria [17], for example, metabolic niche partitioning [18–20], negative frequency-dependent selection [21–23], shared predators or parasites [24,25], and chemical warfare [26–28]. Many of these mechanisms involve tradeoffs between growth rate and other traits, such as the production and/or tolerance of antibiotics, resistance to adverse environments, and predator avoidance.

One understudied tradeoff is the tradeoff between bacterial growth and motility. This tradeoff can result from the metabolic costs of expressing and utilizing propulsion machineries (e.g., flagella) and genetic constrains that limit the simultaneous improvement of growth and motility during evolution [29–34]. The growth-motility tradeoff is interesting because it may help explain and reconcile the apparently conflicting results of a strong tendency of competitive exclusion found in laboratory experiments between competing species of overlapping resource niches and the widespread coexistence of intensely competing strains in nature. In contrast to the often spatially simple and well-mixed conditions in competition assays, natural environments such as the soil, skin, and fermented foods are featured by spatial heterogeneity that allow bacterial cells to interact at different spatial scales, and allow motile strains to disperse. Indeed, studies in animals and plants have shown that the competition-dispersal tradeoff (analogous to the growth-motility tradeoff in bacteria) can contribute to the coexistence of closely related species at relatively large regional spatial scales, but not at the smaller local scales [35,36]. Recent studies with bacteria also showed that a negative correlation between growth rate and dispersal ability can promote coexistence of closely related *Pseudomonas fluorescens* [37] strains, and *Escherichia coli* [32] strains. However, the experimental designs in these studies are highly simplified from biological reality. In the former study [37], the dispersal of bacteria was implemented by

artificially transferring cell suspension across wells in 96-well plates at discrete time intervals; and in the later work [32], a simple and homogenous soft agar gel was used as the substrate where bacterial growth and dispersal took place. Therefore, there is need to test the effects of spatial scales of competition and the growth-motility tradeoff incorporating other relevant biological factors, for example, in spatially heterogeneous environments created naturally by fungal hyphae networks where bacterial cells have the opportunity to disperse by swimming with their flagella actively.

In this work, we study the effect of growth-motility tradeoff in spatially heterogenous environments where the bacteria can disperse through abiotic glass fiber networks or biotic networks that are formed by living fungal mycelia [11,38,39], known as "fungal highways" in the soil environment [38]. Glass fibers have been shown to allow efficient bacterial dispersal and they have been used in several studies to mimic the effect of fungal hyphae in providing a dispersal network for bacteria [39–43]. Therefore, we include the glass fiber network as an abiotic control that can provide dispersal pathways without involving any biotic interactions between fungi and bacteria. We use two motile strains of the bacterium *Pseudomonas putida* to experimentally test whether the spatial scale of interactions affects the competition outcome. Analogous to macro-organisms, the experiments were designed to make the two bacterial strains complete at the local scale when inoculated in mixed cell suspensions, and at the regional scale when inoculated in single-strain suspensions onto the same medium in a Petri dish. In the former case, the cells of the two bacterial strains were intermixed and competed locally right from the start; in the latter case, the cells were distributed in single-strain colonies and the two strains competed for resources that diffused through the medium in the same region (i.e., the Petri dish). While the dichotomy of strict local and regional competition is conceptually easy to understand, the scales of competition in natural environments are often intermediate, due to the dispersal of individuals. To simulate this, we gave the two bacterial strains the opportunity to disperse by swimming with their flagella in the thin liquid layer on the surface of an abiotic or a biotic network that connected different areas in the Petri dish. In the presence of the dispersal networks, the two bacterial strains competed at an intermediate scale that was initially local when inoculated in mixed droplets, and they competed at an intermediate scale that was initially regional when inoculated in separate droplets.

Besides a demonstration of local competition leading to competitive exclusion while regional competition is more favorable for coexistence, our results show that the effect of growth-motility tradeoff on the maintenance of coexistence is contingent on the spatial scale of bacterial competition. In the presence of dispersal, we found that the growth-motility tradeoff helped the slower growing strain to coexist with the faster growing strain only when the initial scale of interaction was regional (when inoculated in separate droplets). When inoculated in mixed droplets with the two strains competing locally directly from the start, the faster growing strain can block the dispersal pathways that can be otherwise taken by the slower growing strain, depriving its dispersal advantage and leading to competitive exclusion. Our study shows that spatial scales of competition and the growth-motility tradeoff interact to determine the competition outcome and the prospect of coexistence of closely related bacterial strains in spatially complex environments.

Materials and Methods

(a) Bacterial and fungal strains

For all experiments, we used two closely related flagellated strains of *Pseudomonas putida*. The KT2440 strain is a saprotrophic soil bacterium, originally isolated from the rhizosphere [44]; the UWC1 strain is a spontaneous mutant of the KT2440 [45]. The KT2440 and UWC1 strains were tagged with the green fluorescent protein (GFP) and mCherry, respectively, by Mini-Tn7 transposon insertions [46,47]. The different fluorescent labels enabled the two strains to be observed by epifluorescence stereomicroscopy and enumerated by flow cytometry. The fungal species used in the experiments was *Trichoderma rossicum* (NEU388) [43], a saprotrophic soil ascomycete that has been shown to allow various bacterial species (including *P. putida*) to move along its mycelial networks.

(b) Culture conditions and inocula preparation

Bacterial cells were cryo-preserved in 30% (v/v) glycerol at -80 °C. To prepare the inocula, cells were first plated on Nutrient agar (NA, 23 g/L, Carl Roth) supplemented with gentamycin (10 ppm) and grown at 30 °C in darkness. The strains were then sub-cultured once on NA through polygonal spreading and grown under the same conditions. Bacterial suspensions were prepared from overnight cultures grown in Nutrient Broth (NB, 25 g/L, Carl Roth) at ambient temperature under constant agitation at 120 rpm. Bacteria were collected from an overnight culture by centrifugation (3000 g for 10 minutes), washed once and resuspended in 0.01 M phosphate-buffered saline (PBS: 1.5 g/L $Na_2HPO_4{\cdot}2H_2O$, 0.25 g/L $NaH_2PO_4{\cdot}2H_2O$, 8,5 g/L NaCl, pH adjusted to 7.4). The cell densities of the two bacterial strains in the suspensions were determined by flow cytometry (see below) and then adjusted to the same value of 10^9 cells/ml. After that, for the local competition scenario, a 1:1 ratio cell suspension was prepared by combining a proportion of the single-strain suspensions.

The fungus *T. rossicum* was maintained on a slanted agar tube at 4°C. It was plated on the malt-agar medium (MA: 12g/L malt extract Pulber amber, SIOS Homebrewing; 15g/L technical agar, BioLife Italiana) and incubated at 22 °C in darkness. Before being used for inoculation, the fungus was sub-cultured by re-plating a

plug cored from the active apical margin of the 2 days old mycelial colony on a fresh medium and grown under the same conditions.

(c) Experimental setup

As shown in Figure 1a, we inoculated the mCherry-labeled UWC1 strain and the GFP-labeled KT2440 strain in droplets of either single-strain or mixed cell suspensions onto plates of MA medium, under three different spatial conditions—no network, fungal mycelial network, and glass fiber network—each with four to eight replicates.

In the "no network" settings, both bacterial strains have limited capacity to disperse. The colonies (both separate or mixed) can only expand from the colony margin (Supplementary Figure S1). Under the "fungal network" and "glass fiber network" settings, the two bacterial strains can use their flagella to swim and thereby disperse in the liquid film along the surface of fungal hyphae or glass fibers, respectively [39].

In the network settings, when the two bacterial strains were inoculated separately, each strain could grow and start to disperse before encountering each other, and therefore the scale of interaction was initially regional. When inoculated in mixed droplets, the two strains engaged in direct competition right from the start before they could disperse, and thus the competition was initially local. Overall, the competition between the two strains occurred at finer scales when inoculated in mixed droplets, and the spatial scale of competition was coarser when the two strains were inoculated in separate droplets.

The bacteria and fungi inocula and the PBS control were positioned according to the diagram in Figure 1b. The distances between the inocula were determined based on preliminary tests. The relatively shorter distances between point A (where the fungi or PBS control were inoculated) and the other inocula ensures a quick start of bacterial-fungal interactions (the fungal mycelium network can grow passed the bacteria inocula within 24 hours, see Supplementary Figure S2). The relatively larger distance between inocula B and D ensures that the separate colonies of the KT2440 and UWC1 strains did not get in direct contact by the end of the experiments (Supplementary Figure S3). We kept the positions of inoculation in Figure 1b fixed throughout all treatments to ensure that the fungal networks were of the same "age" when they expand and enter in contact with the bacterial colonies, because younger and older mycelia behave differently when interacting with bacterial colonies. A mycelium established for a longer timeframe invades bacterial colonies more aggressively (Supplementary Figure S4) and better promotes the dispersal of the KT2440 strain (Supplementary Figure S5).

(d) Inoculation procedures and culture conditions

In the "no network" setting, the inoculations of bacterial suspensions with separate/mixed strains and the PBS control were carried out by dropping on the medium surface 3 µl of the vortexed suspension with a microsyringe (Hamilton® 1701N syringe 10ul needle size 26s ga) according to the corresponding positioning scheme in Figure 1. The resulting inocula were round-shaped with an area of $0.27 \pm 0.011\ \mathrm{cm}^2$.

In the "glass fiber network" setting, we first placed around 30 glass fibers (8 µm diameter, 3-6 cm length, Cole-Parmer, USA) randomly on the agar plate and then performed the inoculations of bacteria as described earlier.

In the "fungal networks" setting, we first inoculated the fungus as a ⌀ 5 mm plug cored with the wide-end of a Pasteur capillary pipette from the distal part of the *T. rossicum* mycelial network. Note that the mycelium was not in direct contact with the medium but laid on top of the plug. The two bacterial strains in separate or mixed droplets were inoculated immediately afterwards according to the schemes in Figure 1, following the same procedures as under the "no network". To prevent the fungal inoculum agar plug from impeding the expansion of bacterial colony/colonies, we removed it after 24 hours, when the mycelial network had already expanded to the medium surface in the Petri dish.

After inoculation, the plates were parafilmed and incubated, in an upright position, at 22 °C in constant darkness.

(e) Collection of bacterial cells for enumeration

After five days of growth, the bacteria cells were collected for enumeration by flow cytometry. Under the "no network" settings, we retrieved all bacterial cells by scraping the whole colony and resuspending the cells in 0.22 µm-filtered PBS. The suspensions were then stored on ice.

Under the "glass fiber network" and the "fungal network" settings, we first removed the bacterial colonies from the plates with a scalpel. We excluded cells in the bacterial colonies because we aimed to examine the relative abundance of the two bacterial strains that had already dispersed in the fungal/glass fiber networks. To collect those cells, we added 7 ml filtered PBS to the Petri dish, closed it, and agitated it at 100 rpm for 20 minutes on a rotary shaker. After that, we intensively flushed the surface of the medium with a 1000 µl micropipette, collected 5 ml of the resulting suspension, and stored it on ice.

All the suspensions were then sonicated in a sonic bath at 35 kHz (Transsonic 310, Elma) twice for 30 seconds each and with a 30 seconds break in between [38]. This step was done to disaggregate bacterial clumps and/or to separate cells that might have stuck to the fungal/glass fiber fragments that might present in the suspension by chance. To remove those fragments before the flow cytometry analysis, we filtered the suspension through a polycarbonate filter with pores of 10 µm (Whatman, Nucleopore Track-Etch Membrane) pre-autoclaved in the Swinnex filters holders. All samples were kept at 4 °C before performing the flow cytometry analysis the day after.

(f) Flow cytometry

We estimated the relative abundances of the bacterial strains with a CyFlow space cytometer (Partec-System) equipped with a blue laser (480 nm/20mV) and a green laser (532 nm/30mV). The FSC and SSC optical parameters are associated with the blue laser. The green fluorescence of the KT2440 strain was excited by the blue laser and detected on a photomultiplier tube with a 488-nm bandpass filter (FL1 parameter). The red fluorescence of the UWC1 strain was excited by the green laser and detected on a photomultiplier tube associated with a 590/50-nm bandpass filter (FL2 parameter). The lasers were arranged in parallel, with a delay of 50 µs between the two signals. Calibration of the instruments was verified before the analysis with calibration beads (Partec-Sysmex). The leading trigger was set on SSC, with a lower threshold of 500. Gains were set to 700 for FL1 and 750 for FL2. A four-decade logarithmic amplification was used for all parameters. The instrument was equipped with a true volumetric absolute counting facility: events were counted in a defined volume of 200 µl. A speed of 2 µl/s was used. We diluted the samples with 0.22 µm-filtered PBS to reach an event rate of ~1500 total events/s. This rate was determined with preliminary assays to yield an optimal signal to noise ratio. Samples were kept on ice during the whole analysis and were run in random order. Analysis of the flow cytometry data was performed with the instrument software FloMax. Events corresponding to the mCherry and GFP fluorescent signals were selected and counted with a quadrant gate set on a biplot of the FL1 and FL2 parameters.

(g) Data preparation and statistics

We focused on the relative abundance of the UWC1 and KT2440 strains, and therefore, we calculated the cell density ratio in each replicate at the end of the experiments. We then transformed the data by taking the common log (of base 10) and confirmed the normality of the transformed data with the Shapiro-Wilk normality test. We then performed the Student's t-test against our null hypothesis that the relative abundance of the two strains remains identical to that at the beginning of experiments (with a log ratio of zero). The competitions between the abundances of the two strains when inoculated in mixed or separate droplets were performed by the Welch's two sample t-tests. The statistical significance threshold value was chosen at $p = 0.05$.

Results

Under the "no network" treatment, when bacteria cells were inoculated in separate droplets (corresponding to regional competition), the relative abundance of the KT2440 strain and the UWC1 strain remained the same after 5 days of growth (Figure 2a, Tables 1 and 2). In contrast, in mixed inocula (corresponding to local competition) the UWC1 strain reached much higher cell densities at the end of the experiments (the UWC1 strain was on average 5.43 times more abundant than the KT2440 strain), showing a growth advantage of the UWC1 strain in direct competition. Moreover, the spatial distribution of the two fluorescently tagged strains indicated that the UWC1 strain has expanded faster and constrained the KT2440 strain to the interior of the mixed colony, thus blocking the further growth of the KT2440 strain (Figure 2b). The marked difference in the relative competitiveness of the two strains implies competitive exclusion of the KT2440 strain by the UWC1 strain at the local scale in the long term.

Under the "glass fibers" treatment, the KT2440 strain tended to be slightly more abundant in plates where the two bacterial strains were inoculated separately (corresponding to an intermediate scale of competition that was initially regional, see Figure 2a, Tables 1). It reached on average 1.24 times the cell density of the UWC1 strain, indicating a motility advantage of the KT2440 strain in the presence of a dispersal network, but with large dispersion of the measurements between replicates. This variation is probably caused by the randomness of the dispersal network that is created when placing the extremely thin (8 $\boldsymbol{\mu m}$ in diameter) and brittle glass fibers on the Petri dish (see Supplementary Figure S6 for picture of a glass fiber network on a Petri dish). We therefore cannot reject the null hypothesis that the relative abundance of the two strains remained identical (Table 2). This result implies possible long-term coexistence of the two strains at the regional scale. In plates where the two bacterial strains were inoculated in mixed droplets (corresponding to an intermediate scale of competition that was initially local), similar to the results of local competition under the "no network" treatment, the UWC1 strain has a substantial competitive advantage and reached on average 6.99 times the density of the KT2440 strain, implying that competitive exclusion is likely at the local scale (Figure 2a, Tables 1 and 2).

Under the "fungal network" treatment, the KT2440 strain reached even larger (statistically significant) relative abundance when inoculated in separate droplets (1.42 times the abundance of the UWC1 strain), implying a competitive advantage at the regional scale. The competitive advantage of the KT2440 strain outside the initial inocula resulted from its higher motility and faster spread in the presence of fungal hyphae networks. The distribution of bacterial cells in the fungal network can be considered as mini-colonies with either mixed or separate strains. Since the KT2440 strain grows equally fast as the UWC1 strain in separate colonies, and it grows much slower than the UWC1 strain in mixed colonies, the overall higher densities of the KT2440 strain can only be due to its superior motility in the presence of dispersal networks provided by fungal hyphae. In mixed inoculations, however, the KT2440 strain was competitively excluded outside the initial inocula (Figure 2c for an example) in two of the replicates (in these cases, the cell densities of the KT2440 strain were so low that they were not distinguishable from the noise background in the flow cytometry assays), and in the other two

replicates, the cell densities of the UWC1 strain was 3.72 time and 10.67 times more abundant than the KT2440 strain (Figure 2a, Tables 1 and 2). The spatial distribution of the two tagged strains indicated that when inoculated in mixed droplets in the presence of fungal networks, the slower growing KT2440 strain was constrained to the interior of the mixed colony, and consequently, was not able to disperse through the fungal networks (Figure 2c, note that only red fluorescence was visible along the fungal hyphae connected to the colony). In contrast, when inoculated in separate droplets, both bacterial strains can efficiently disperse along fungal hyphae networks (Figure 2d-e).

Across all spatial settings, the relative abundance of UWC1 and KT2440 strains differ greatly depending on whether they were inoculated in separate or mixed droplets (Figure 2a, Table 3), showing the importance of the spatial scale of competition in determining the competition outcome and long-term coexistence of the two bacterial strains, in the presence or absence of dispersal networks.

Discussion

Our experimental results showed a clear pattern: local competition between the two bacterial strains (treatments with mixed inocula) caused competitive exclusion, while competition at the regional scale (treatments with separate inocula) is more favorable for coexistence. This is consistent with the effect of the spatial scale on the competition outcome found in larger organisms [35,36]. Our results thus help to reconcile the conflicting findings that bacteria [6–8] and protists [48] species/strains that overlap in their niches of resource utilization tend to competitively exclude each other in well-mixed liquid cultures (implying local competition), and the widespread phenomenon of microbial species/strains coexisting while competing for the same resources in spatially complex environment (implying regional competition), such as cheese rind [11,12], the human gut [13,16] and skin [14]. It is important to note that spatial complexity does not automatically promote biodiversity. The effect of spatial structure crucially depends on the type of interactions between species, and in many cases, adding spatial structure can lead to the breakdown of diversity and competitive exclusion of species that could have coexisted under a well-mixed environment. For example, when species interact via metabolic mutualism (cross-feeding), spatial structure can hinder the exchange of metabolites due to reduced diffusion, thus causing the loss of biodiversity [49]. When species interactions involve metabolic tradeoffs, spatial structure may also reduce biodiversity due to unutilized resources by the local species diffusing to and being used by the more competitive neighboring species [50]. The recent work of Lowery and Ursell (2019) [51] provided a pair of contrasting examples to illustrate how the effect of spatial structure can change depending on the mechanistic details of interspecific interactions. The authors showed that by adding spatial structure, the coexistence of three species that exhibit cyclic dominance competition (e.g. as in a children's "rock-paper-scissors" game) may become more likely to collapse, while the coexistence of two mutually killing species that otherwise competitively exclude each other can be maintained.

In the current work, we showed that when the competing species are involved in a growth-motility tradeoff, spatial structure provided by dispersal networks can help the competing species to coexist. In the presence of dispersal networks, the higher motility of the slower growing strain (KT2440) can confer an advantage only when the initial scale of competition was regional (the two strains were inoculated in separate droplets). Otherwise, the faster growing strain (UWC1) can quickly outgrow the slower growing strain, constraining the latter to the interior of the mixed colony, and thus preventing it from dispersing. In particular, instead of comparing the growth rates of the UWC1 and KT2440 strains in isolation, we inferred the growth advantage of the UWC1 strain in direct competition by comparing the log ratios of the cell densities under mixed and separate inoculations in the "no network" setting, controlling that spatial arrangement was the only difference. We also confirmed that the UWC1 strain constrained its disadvantaged competitor to the interior of mixed colonies by fluorescence microscopy. Microbial growth rates are highly context-dependent. Literature has shown that even when growing in monoculture the relative growth rates of competing species can switch in different environments (e.g. *in vivo* in the host environment and *in vitro* when cultured in microcosms [52]), and the relative growth rates in coculture can show complex non-linear patterns with multiple crossings at different initial frequency combinations [22]. Not only the relative growth rates of competing microorganisms may switch, the absolute growth rates can also change across large magnitudes. For example, by changing the number and species of co-culturing microorganisms, the growth rate of a dinoflagellate can vary across four-folds, equivalent to the effect of a 5 °C change in temperature or an almost six-fold change in light intensity [53]. Precisely due to the sensitivity of microbial growth rate on the biotic and abiotic environments, new methods and devices have been developed to improve the accuracy and convenience of measuring growth rates in coculture [54,55]. Furthermore, it was recently found that microbial motility may depend on growth rate due to a tradeoff in resource allocation, as *E. coli* cells growing in nutritionally poor conditions invested more in motility in proportion to reproduction, probably as an anticipation of the benefit from dispersal [56]. Because of the strong association between microbial growth and dispersal rates and their sensitivity to biotic and abiotic environmental factors, it is important to measure them in the same competition context, as we did in the current work.

Note that the significantly higher abundance the KT2440 strain in the presence of fungal networks when inoculated in separate droplets does not imply that it can competitively exclude the UWC1 strain because of two reasons. First, we focused on quantifying the relative abundance of cells *outside* the colonies formed by the initial inocula, and thus the result of higher abundance of the KT2440 cells was limited to cells descended from

the dispersed ones, and it is possible that the UWC1 strain has nevertheless reached higher abundance *inside* the initial colony. Second, our results have shown consistent growth advantage of the UWC1 strain when competition occurs locally. Therefore, we expect that as soon as the cells of the two strains meet in the dispersal network and engage in direct local competition, the UWC1 strain would overgrown its competitor locally. The competitive advantage of the UWC1 strain in local competition is highly contingent on the exclusive spatial occupation of cells. Unlike diffusible chemical-based nutrients, space is a non-sharable resource. By occupying specific locations in space, such as the expansion frontiers of a mixed bacterial colony or the locations where a mixed colony meets a dispersal networks, a faster-growing species (the UWC1 strain in our case) not only prevents its competitor from occupying the same space per se, but also deprives it the opportunity to use a section of space extending from the colony margin [57–59], and in the presence of a dispersal network, also the section of space in the network that is accessible only through that location. Existing work has shown that physical interactions, including the steric repulsive forces between neighboring cells, play important roles in the morphogenesis of micro-colonies [60], shaping the structure and community dynamics of bacterial biofilms (see a recent review of [61] and references therein), interacting with the spatial structure of the substrate to affect bacterial competition [51,62], and stochastically alter the trajectories of neutral evolutionary dynamics in spatially heterogeneous environments [63]. In particular, a recent work integrating experiments and modelling showed that the radial expansion of bacterial colonies is not limited by nutrients as commonly believed, but by mechanical forces through space competition; while nutrient penetration governs vertical colony growth instead [64]. In this work, we showed that the spatial occupation of cells at the "entrance points" of a dispersal network not only has an effect at present, but also gives the cells at those locations an "opportunity advantage" to explore and expand to new territories in the future.

The competitive advantage of the KT2440 strain when dispersal is allowed (significant under the fungal network treatment, and tendential under the glass fiber network treatment) suggests that the growth-motility tradeoff plays a role in promoting the coexistence of the two bacterial strains, with the UWC1 strain growing faster under direct competition while the KT2440 is better at dispersing and exploring empty habitats. When competing species (not necessarily microorganisms) are regulated by a tradeoff between their rate of reproduction and their ability to disperse, theory predicts that there are conditions where coexistence is possible [65,66]. The tradeoff has been found in sympatric bacterial strains [32,34] and can be controlled, for instance, by the ability to secrete extracellular polymeric substances for biofilm formation [33]. The effect of the tradeoff in promoting coexistence has been shown in an experimental system with two *P. fluorescens* strains, where the authors manipulated the dispersal rate of each bacterial strain by transferring them at different relative abundances to wells in 96-well plates [37]. The competition within each well in the 96-well plates corresponds to local competition, while the coexistence of different strains across the 96-well metapopulation corresponds to regional competition. Their results are, therefore, consistent with ours.

In the aforementioned study [37], the dispersal events occurred, however, by manually transferring bacterial cells at discrete intervals. Such experimental design, while promoting the controllability of the system and helping to reduce variation between different replicates, is highly artificial and corresponds to natural conditions that only rarely occur (e.g., occasional heavy rains that saturate the soil for a short time [47,67,68]). In this work, we allowed continuous dispersal of the two bacterial strains, either through abiotic and static glass fiber networks, or growing fungal mycelial networks that can interact dynamically with the bacteria [69,70]. The bacterial dispersal in our experiments thus corresponds to that in environments such as the typical well-drained soil, where the movement of bacteria is restricted by the thinness and patchiness of the liquid films [71] on the surface of biotic (e.g., roots, fungal hyphae) and abiotic (e.g., rocks and soil particles) components. Despite the additional factors and variation involved, our results showed robustly that interactions at the local scale tend to cause competitive exclusion, while coexistence is more likely when competition is regional.

In this work, the abiotic network was formed by placing thin glass fibers randomly on a Petri dish, while the biotic network was formed by the actively growing fungal hyphae, which follow species-specific structural patterns. Theory and experiments have shown that the network structure and connectivity can shape biodiversity patterns [72,73]. For example, dispersal along dendritic landscapes can lead to higher variability in local diversity and among-community composition [74]. Bacterial dispersal along abiotic glass fiber network and living fungal hyphae network is, therefore, likely to be affected by the topology and temporal dynamics of the network, and dispersal is likely to be directionally biased due to density effect and/or resource gradients. Compared to glass fiber networks that only provide a physical pathway for rapid displacement of bacterial cells, the biotic interactions between fungi and bacteria, e.g., via chemical signaling and predation [69,75], may also affect bacterial dispersal and their population dynamics along fungal hyphae networks. Since we were not able to precisely control the network structures in the current work, large variations persist in the relative abundance of the two bacterial strains, especially under the experimental settings with glass fiber networks. This also limits our ability to investigate deeper into the interactions between network type and different ways of inoculation, for example, by performing a two-factor ANOVA test. Therefore, a valuable next step would be to tease apart the large variations of our current results under the abiotic and biotic network settings by manipulating their structures, for example, by using 3D printed abiotic hyphae models with fixed structure and surface physicochemical properties, or microfluidic platforms that constrain the growth of fungal hyphae networks into pre-defined topology [76,77].

Conclusion

To conclude, using experiments with two closely related strains of the bacteria *Pseudomonas putida*, we found that spatial scales of competition and the growth-motility tradeoff interact to determine the competition outcome. We showed that local competition caused competitive exclusion while regional competition is more favorable for coexistence. The results are consistent with previous findings in animals and plants. Furthermore, we showed that in the presence of dispersal networks, the growth-motility tradeoff can promote coexistence only when the scale of competition was regional initially. Only in this case, the slower growing but faster dispersing strain has the opportunity to use the dispersal networks to escape from direct local competition with its faster growing competitor. Our conclusions are likely to generalize to microbial competition dynamics in spatially complex environments where interactions occur at different spatial scales and in the presence of dispersal networks. We thus encourage future work to systematically study the interactions between spatial scales of competition and the growth-motility tradeoff between pairs of microorganisms with different phylogenetic relatedness. The current work identified new factors that affect microbial competition dynamics in spatially complex environments — in particular, along dispersal networks provided by fungal hyphae, and raised new questions regarding the roles of network topology and the biotic interactions between fungi and different bacterial strains that disperse along them. Future investigations along these lines would help us delve deeper into the mechanisms that govern microbial competition dynamics and coexistence in spatially complex environments.

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Ethical Statement

Not applicable.

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Data Accessibility

All data supporting the reported results can be found in the data tables in the manuscript and the Supplementary Materials.

Competing Interests

We have no competing interests.

Authors' Contributions

P.J., R.B, S.B. conceived the research project, P.J., R.B., X.L., S.B., D.G. conceptualized the experiments, M.M. and T.K. developed the methodology, performed the experiments and analyzed the data, A.E. and F.P. provided supports to the methodology, A.E. and D.G. helped with the flow cytometry assays, X.L. wrote the manuscript with inputs from all authors.

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Tables

	No network	Glass fiber network	Fungal network
Separate inoculation	1.064	1.239	1.418
Mixed inoculation	0.184	0.143	N.A.[1]

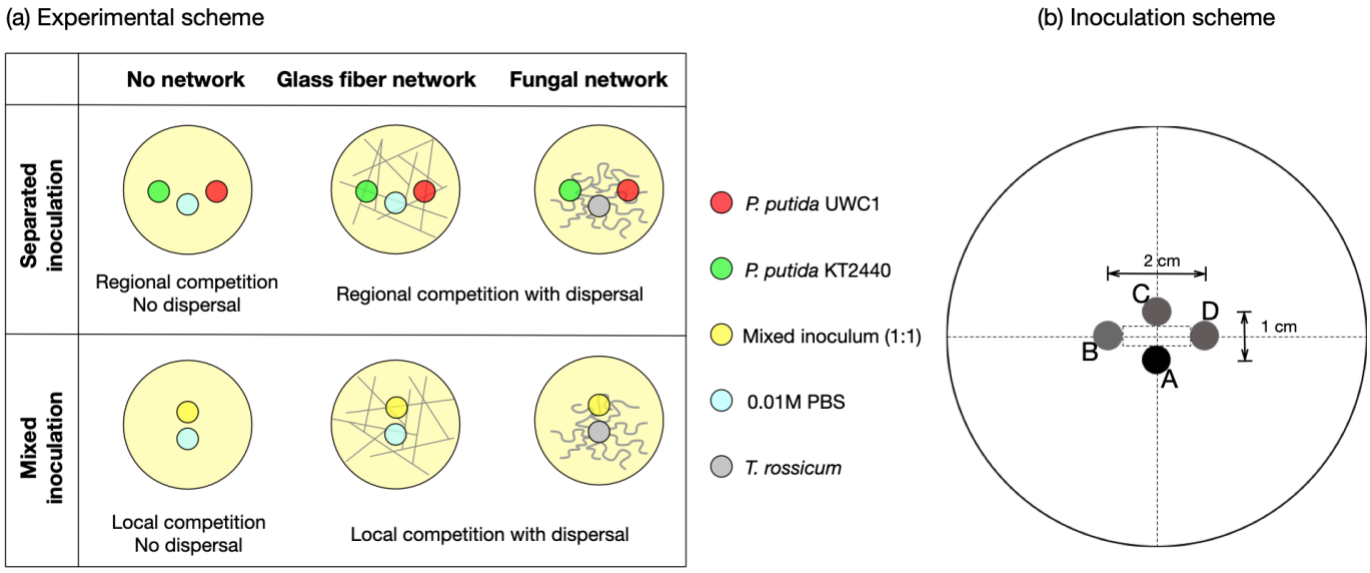
[1] The KT2440 strain was competitively excluded in two of the four replicates.

	No network	Glass fiber network	Fungal network
Separate inoculation	0.030 ± 0.094 ($\boldsymbol{p}$=0.455)	-0.006 ± 0.688 ($\boldsymbol{p}$=0.978)	0.170 ± 0.111 ($\boldsymbol{p}$=0.017)
Mixed inoculation	-0.746 ± 0.039 ($\boldsymbol{p}$<0.01)	-0.820 ± 0.488 ($\boldsymbol{p}$=0.013)	N.A.[1]

[1] The KT2440 strain was competitively excluded in two of the four replicates.

Spatial settings	p-value of the Welch's two sample t-test
No network	$\mathbf{2.35 \times 10^{-7}}$
Glass fiber network	0.025
Fungal hyphae network	N.A., because of the two $-\infty$ values under mixed inoculation treatment

Figures



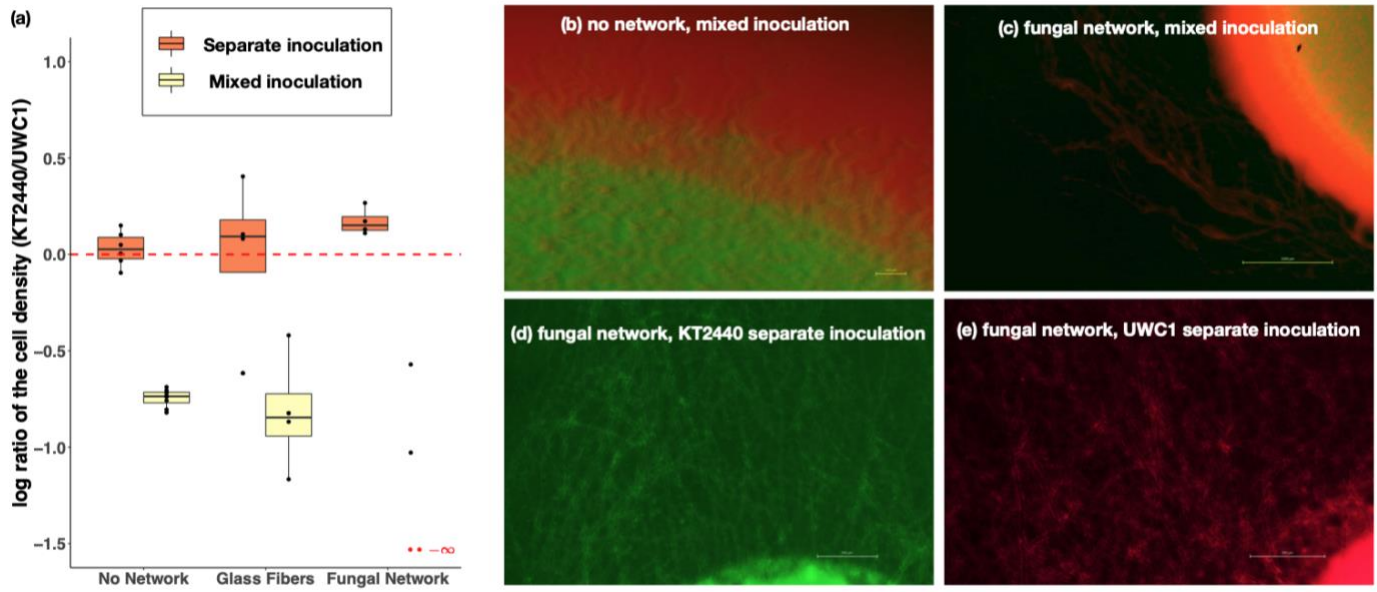


Figure and table captions

Table 1. The median of the absolute density ratio (before log transformation) of the KT2440 strain relative to the UWC1 strain.

Table 2. Mean and the standard deviation of the log density ratio between the KT2440 and the UWC1 strain, and the ***p***-value of the t-test with the null hypothesis that the mean of the log-ratio was not significantly different from zero.

Table 3. Comparison between the distributions of the common logarithm (base 10) of the cell density ratio (KT2440/UWC1) under separate and mixed inoculation treatments.

Figure 1. (a) Diagram of the experimental setup under spatial settings representing different spatial scales of competition. The inocula is represented in the diagram as disproportionally large for the purpose of a clear illustration. The diameters of the bacteria/PBS inocula and the fungal inocula were 5.8 mm and 5.0 mm, respectively. See panel (b) for a diagram of the actual positions of different inoculates on a ⌀ 9 cm Petri dish. When applicable, the fugus or the PBS control were inoculated at position A, the KT2440 strain suspension was inoculated at position B, the mixed inoculum was inoculated at position C, and the UWC1 strain was inoculated at position D.

Figure 2. Box-Whisker plots representing the distribution of the common logarithm (base 10) of the cell density ratio (KT2440/UWC1) under different experimental settings. The boxplot center lines show the median, box limits show upper and lower quartiles, whiskers show 1.5x interquartile range, and each point corresponds to the result of an independent replicate. The red dashed line represents equal cell densities of the two bacterial strains. The numbers of replicates were 6 and 8 under the "no network" setting for the separate and mixed inoculations, respectively. Under all other settings, the numbers of replicates were 4. Competitive exclusion of the KT2440 strain by the UWC1 strain occurred in two replicates under the fungal network treatment with mixed strain inoculates (the log density ratio was $-\infty$, marked with the two red dots). (b) Colony formed by bacterial strains in a mixed droplet two days after inoculation in the absence of a dispersal network. (c) Colony formed by bacterial strains in a mixed droplet in the presence of fungal hyphae networks two days after inoculation. Only the UWC1 strain with red fluorescence was visible along the fungal hyphae. (d) Dispersed KT2440 cells along fungal hyphae networks three days after being inoculated in a separate droplet. (e) Dispersed UWC1 cells along fungal hyphae networks two days after being inoculated in a separate droplet. The pictures were taken at magnifications of 10x, 3x, 5x, and 5x with epifluorescence stereomicroscopy in panels b, c, d and e, respectively.