

ORIGINAL ARTICLE

Catch me if you can: dispersal and foraging of *Bdellovibrio bacteriovorus* 109J along mycelia

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To cope with heterogeneous environments and resource distributions, filamentous fungi have evolved a spatially extensive growth enabling their hyphae to penetrate air–water interfaces and pass through air-filled pores. Such mycelia are also known to act as dispersal networks for the mobilisation of bacteria ('fungal highways') and connection of microbial microhabitats. Hitherto, however, nothing is known about the effect of mycelia-based dispersal on interactions between bacterial predators and their prey and concomitant effects on biomass formation. We here hypothesise that mycelia enable the contact between predators and their prey and shape a prey's population. We investigated the impact of predation by *Bdellovibrio bacteriovorus* 109J on the growth of its potential prey *Pseudomonas fluorescens* LP6a in the presence of mycelia. Our data give evidence that hyphae increase the accessibility of the prey to *B. bacteriovorus* 109J and, hence, allow for efficient foraging and shaping of prey populations not seen in the absence of mycelia. To test our hypothesis tailored microbial landscapes were used for better reduction of emerging properties in complex systems. Our data suggest that mycelia have substantial influence on prey–predator relationship and hereby may promote the structure of prey and predator populations and, hence, may be a determinant for biomass formation in heterogeneous environments.

The ISME Journal (2017) 11, 386–393; doi:10.1038/ismej.2016.135; published online 8 November 2016

Introduction

How can a water-bound predator reach a prey item, which is separated by an air-filled soil pore? Whereas various aspects of soil heterogeneity, such as the transport and distribution of resources as controls of microbial activity and biomass formation, have been dealt with in detail (Bosma *et al.*, 1997; Johnsen *et al.*, 2005; Harms and Wick, 2006; Semple *et al.*, 2007), restrictions to the physical interaction between micro-predators and their prey in poorly permeable environments have only scarcely been addressed (Yair *et al.*, 2003). Recently, it was discovered that fungal mycelia act as networks ('fungal highways'), along which bacteria can disperse and even pass through air-filled parts of water-unsaturated environments (Kohlmeier *et al.*, 2005; Wick *et al.*, 2007b). Several studies demonstrated a positive effect of bacteria–fungi interactions on ecosystem services such as increased transformation of anthropogenic and natural compounds in soil

(Boonchan *et al.*, 2000; Warmink *et al.*, 2011; Banitz *et al.*, 2011b; Pion *et al.*, 2013a). However, bacterial dispersal along mycelia could also evoke antagonistic effects such as the mutual inhibition or competition between bacteria and fungi (Rousk *et al.*, 2008; Pion *et al.*, 2013b).

In the present study, we tested whether bacterial predators can use mycelia as means to reach their prey and whether there are consequences for the prey population. As environmental conditions (for example, the availability of carbon sources) are known to trigger tactic bacterial dispersal (Furuno *et al.*, 2010; Krell *et al.*, 2012; McDougald *et al.*, 2012; Son *et al.*, 2015), we further approximated if a predator's dispersal may be directed by the presence of bacteria. *Bdellovibrio*-and-like-organisms (BALOs) are the best studied bacterial predators. The biphasic life cycle of BALOs consists of a motile free-living phase, in which the cells are searching and attacking the prey, and a growth phase within the periplasm of their Gram-negative prey (bdelloplast), during which multiple progeny are produced before they escape after the host has been consumed (Stolp and Starr, 1963, 1965; Sockett, 2009). Because of their exceptional motility and unique life cycle, BALOs can control prey populations so effectively that they even hold promise as antibacterial agents in food industry, agriculture or medicine (Markelova, 2010; Dwidar

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Received 21 June 2016; revised 17 August 2016; accepted 29 August 2016; published online 8 November 2016

et al., 2012; Johnke et al., 2014). From medical studies, for instance, it is known that *Bdellovibrio bacteriovorus* is able to attack and destroy oral multilayer biofilms (Dashiff and Kadouri, 2011; Dashiff et al., 2011). The role of predatory bacteria in the microbial ecology of heterogeneous subsurface environments, including the control of population size and structure of Gram-negative bacterial populations, which often live and grow in association with surfaces, is less clear (Markelova and Colwell, 1999; Dashiff et al., 2011). For our study, a controlled microcosm system as a mimic for an air-filled porous system was tailored. Mycelia were allowed to bridge physically separated predator and prey populations, and the impact of predation on the prey population was assessed. Our data show that BALOs disperse along mycelia and that this movement is consequential for the prey populations.

Materials and methods

Bacteria strains, media and growth conditions

Pseudomonas fluorescens LP6a was used as the prey bacterium in dispersal and chemotaxis experiments (Foght et al., 1998). It was grown at 28 °C on a rotary shaker at 150 rpm in Erlenmeyer flasks containing 200 ml of 1:10 diluted nutrient broth (1:10 dNB). For microcosm experiments, 24 h old cultures were harvested in the exponential phase, washed twice in 50 mM potassium phosphate buffer (PBS) of pH 7.2 and centrifuged at 1000 g for 10 min. The pellet was re-suspended in PBS containing $\approx 10^9$ cells per ml. Cell numbers were counted with a MoFlo flow cytometer (DakoCytomation, Fort Collins, CO, USA) as described earlier (Hübschmann et al., 2007). The instrument was adjusted using fluorescent 1- μ m beads and cell counting was performed with 488 nm light of 400 mW to analyse the forward light scatter and the side light scatter.

Escherichia coli ML-35 was used as the prey bacterium for plaque-forming units (PFU) quantification as well as in chemotaxis experiments. It was grown over night at 30 °C on a rotary shaker at 150 rpm in Erlenmeyer flasks containing 200 ml of 1:10 dNB. *B. bacteriovorus* 109J was routinely grown as plaques in double-layered 1:10 diluted nutrient broth (1:10 dNB) agar, amended with 3 mmol l⁻¹ MgCl₂ × 6H₂O and 2 mmol l⁻¹ CaCl₂ × 2H₂O (pH 7.2) and agar (0.7% w/v agar in the top layer). To initiate a lysate, a plug of agar containing *B. bacteriovorus* 109J was added to washed prey cells of *P. fluorescens* LP6a in Erlenmeyer flasks containing 200 ml 1:10 dNB and incubated at 30 °C on a rotary shaker at 150 rpm for 72 h. To harvest the predators, co-cultures were prepared, in which 3 ml of washed host cells were incubated with 600 μ l of stock lysate of *B. bacteriovorus* 109J in 150 ml of 1:10 dNB. The co-cultures were incubated for 48 h to reach a final concentration of $\approx 10^8$ cells per ml of the predator and the suspension was passed three times through a

0.45 μ m Millex pore-size filter (Millipore, Billerica, MA, USA) to remove residual prey and cell debris (Dashiff et al., 2011). Prior to the experiments the initial cell densities of *B. bacteriovorus* 109J were quantified by microscopic counting of DAPI-stained cells. For that, sodium azide (10% (v/v)) fixed aliquots were filtered onto 0.2 μ m Millipore filters (type GTTP) and DAPI-stained (final concentration 12.5 μ g ml⁻¹, diluted with Citifluor AF1). Cell densities were determined by counting five randomly selected fields with the Nikon macroscope (AZ 100 Multizoom) equipped with an Hg vapor lamp and a black-and-white camera (Nikon, Amsterdam, Netherlands) and using the GFP-filter settings. Mycelia of the oomycete *Pythium ultimum* (Maurhofer et al., 1992) were used as model dispersal networks. They were pre-grown at room temperature on potato dextrose agar (PDA; 1.5% w/v; pH 6.8, Difco Laboratories, Franklin Lakes, NJ, USA) (Schamfuss et al., 2013).

Dispersal experiments

Dispersal experiments were conducted at least in triplicate in a microcosm mimicking surfaces of soil constituents under predation pressure and in the presence of a dispersal network (Figure 1). The basic body of a microcosm consisted of an agar strip of 1:10 dNB (length: 3.6 cm, height: 1.0 cm, width: 1.0 cm and agar 1.5% w/v). As illustrated in Figure 1, the agar strip was divided in three zones of interest (A, B and C, length: 1.2 cm, each). An agar cube (1 cm³, minimal medium agar, 2% w/v) was placed next to the agar strip as a starting point for the growth of the *P. ultimum* mycelium and dispersal of the predator *B. bacteriovorus* 109J. The microcosms were inoculated with *P. ultimum* and incubated for 18 h to allow it to grow over the air gap and into the initial zone of the agar strip. Then, the left-hand third of zone A of the microcosms was inoculated with 2 μ l (containing 2×10^6 cells) of suspended *P. fluorescens* LP6a. The microcosms were again incubated for 24 h to allow dispersal of prey cells along the mycelia prior to the inoculation with 2 μ l (containing 8×10^3 cells) predator suspensions on the agar cube and incubation for additional 72 h. All microcosm setups were placed in standard glass petri dishes and stored at 25 °C in a 30-l desiccator.

After 24, 48 and 72 h, the zones A–C of individual microcosms were harvested separately and the number of predator and prey bacteria quantified. For that the agar piece was cut with a sterile spatula and the agar pieces successively suspended in 3 ml PBS, vortexed for 1 min, sonicated (2 × 30 s with a break of 1 min), and their bacterial prey count analysed by colony-forming units. To quantify the predator, a double-layer procedure was used and PFU counted: a suspension of *E. coli* ML-35 cells in soft agar serving as indicator prey was poured on top of agar on which predator cells had been spread. Within 5–7 days incubation, PFU of 2 mm diameter

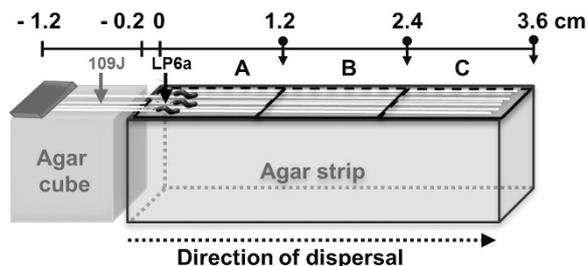


Figure 1 Microcosms used to study the effect of predation of *Bdellovibrio bacteriovorus* 109J on dispersal of *Pseudomonas fluorescens* LP6a along mycelia of *P. ultimum* that was inoculated on an agar block separated by an air gap of 0.2 cm from the agar strip. Prey (black arrow) and predator (grey arrow) bacteria were spot-inoculated consecutively after 24 h on the top of the agar strip and an agar block separated by an air gap of 0.2 cm from the agar strip. *P. ultimum* provided dispersal networks for both bacterial strains. It was inoculated as a freshly overgrown 1:10 dNB agar piece top-side down on the left-hand third of the agar cube. Identical setups without bacterial predator and without prey bacteria, respectively, served as controls. A full colour version of this figure is available at the *ISME* journal online.

developed, which were counted to quantify predator concentrations. Controls were performed without mycelia as dispersal network (in order to correct for potential air-borne transport of the predator; control I), without predator (in order to assess dispersal and growth of the prey bacteria in the absence of predation; control II), and without the prey (in order to estimate dispersal and growth of the predator in the absence of the prey; control III). To rule out possible negative biotic effects of mycelia, experiments with glass fibres (~300 pcs, length: 5 cm with a diameter of ~8 µm) as abiotic dispersal networks were tested in presence (control IV) and absence of prey cells (control V). To evaluate dispersal of the predator along the agar surface in the absence of mycelia, *B. bacteriovorus* 109J cells were inoculated at the left-hand third of zone A (Figure 1) and LP6a prey cells were placed at a distance of ca. 1.2 cm in zone B of the microcosms (control VI). The setups were incubated for 72 h at 26 °C and the number of predator cells quantified as PFU on zone B and C after 48 h and 72 h. All controls were performed at least in triplicate using (where applicable) 2 µl of a *B. bacteriovorus* 109J suspension (1×10^4 cells) and 2 µl of *P. fluorescens* LP6a (2×10^6 cells) as inocula.

Assessment of directed dispersal of predator towards prey cells

A modified capillary test described by Adler (1973) was used to assess possible directed dispersal of *B. bacteriovorus* 109J towards *P. fluorescens* LP6a (Supplementary Figure SI1; Adler, 1973; Straley and Conti, 1974). In short, a culture of *B. bacteriovorus* 109J exponentially growing on *P. fluorescens* LP6a was harvested, centrifuged at 1000 g for 10 min, filtered through a 0.45 µm pore-size Millex filter and re-suspended in HEPES metals (HM) buffer (Ferguson *et al.*, 2014). About 0.3 ml of this

suspension was placed in a small chamber formed by a U-shaped capillary placed on a microscope slide and closed with a glass coverslip in order to prevent the formation of air bubbles within the chamber. Two capillary tubes with volumes of 2 µl (BRAND GmbH & Co KG, Wertheim, Germany), heat-sealed at one end, containing either living prey cells from the late exponential phase, lysed prey cells (heated in a microwave oven at 600–700 W for 60 s) or a mixture of both, were exposed to the predator suspension with their open ends. Furthermore, to determine the predator's response to the supernatant of *P. fluorescens* LP6a, late exponential prey cells of strain LP6a were removed by centrifugation (1000 g for 20 min) and the supernatant was filter sterilised. Identical setups with capillaries containing HM buffer and 1:10 dNB medium served as controls. The chambers were incubated for 20 min (that is, below reported generation times of 2–3 h (Sockett, 2009)) at room temperature and the number of predator cells immigrated in test capillaries was quantified as PFU. All assays were installed in triplicate using two capillaries per setup.

Predation impact

An adaptation of the unitless predation impact (PI) approach by Fox (2002) was used to approximate predation effect of *B. bacteriovorus* 109J on the population size of *P. fluorescens* LP6a:

$$PI = \frac{K_{\text{bac}} - R}{K_{\text{bac}}} \quad (1)$$

Here K_{bac} is the prey cell number at a given time within the system in the absence of predation and R is the corresponding prey cell number remaining under predation pressure. The PI varies between 0 (no effect of predation) and 1 (complete extinction). In case of predation leading to increased abundance of the prey, the value of PI becomes negative.

Statistical analysis

Unless otherwise stated, results of the replicate series for microcosms were compared with each other using a two-sided Student's *t*-test to test for significant differences (**P*-value = 0.05; ***P*-value = 0.005; ****P*-value = 0.001). The variance of absolute quantitative measures is given by the s.d. as depicted in all figures by vertical whiskers. In contrast, the level of uncertainty of relative measures is expressed by reporting 90% confidence intervals (CI) as calculated according to Fieller's method (Fieller, 1940) using the free online calculator of GraphPad (GraphPad Software, San Diego, CA, USA).

Results

Effect of mycelia on dispersal and abundance of *B. bacteriovorus* 109J

Dispersal of *B. bacteriovorus* 109J along mycelia and its influence on the prey organism *P. fluorescens*

LP6a were analysed in microcosms (Figure 1) in presence and absence of mycelia of *P. ultimum* serving as bridging networks. In the absence of mycelia (yet in presence of bacteria; control I) no predator cells (0 PFU) were detected beyond the air gap after 24, 48 and 72 h. In presence of mycelia by contrast, 75 ± 25 PFU ($\approx 1\%$ of the inoculum; Figure 2d) were detected on the agar strip beyond the air gap after 24 h of co-incubation with 17 ± 14 PFU and 8 ± 14 PFU in zones B and C (Figures 2a–d). After 72 h and as a result of growth $4.6 \times 10^6 \pm 1.8 \times 10^6$ PFU (that is, a 6×10^4 -fold increase (CI: 2.9×10^4 – 1.2×10^5)) of the predator (Figure 2d) were seen. If mycelia yet not bacteria were present (control III), no predator cells were detected at any time beyond the air gap neither by PFU counting (0 PFU) nor by microscopic screening of zone A. In order to rule out biotic effects of mycelia, transport experiments with abiotic dispersal networks were tested in presence (control IV) and absence of prey cells (control V). As with mycelia high abundance of the predator in zone A in presence (control IV) yet not in the absence of prey cells (that is, 0 PFU; control V) was detected.

Effect of predation on abundance of P. fluorescens LP6a

The effect of dispersal and abundance of *B. bacteriovorus* 109J along mycelia was evidenced by quantifying the number of prey *P. fluorescens* LP6a cells in zones A–C in presence and absence of predators (Figure 2). In order to avoid starvation of the prey, a mildly nutrient-enriched (that is, 1:10 dNB) environment was provided. Regardless of the presence of active predators dispersing along mycelia, LP6a cells were detected in all three zones with declining cell densities from zone A to C at all times (Figure 2). In zone A, despite constantly rising predator numbers, predation resulted in a

statistically not significant increased prey biomass (Figure 2a) as reflected by a $PI < 0$ (Figure 3). In the other zones, by contrast, prey populations decreased statistically significant by 12–22-fold (zone B) and 15–80-fold (zone C) relative to a predator-free control during the experiment (Figures 2b and c). This also led to a 43% (CI: 34–52%) decrease of the overall prey’s population size (zone A–C) and simultaneous shift of the prey-to-predator ratio from 1.3×10^3 (CI: 106–1555) to 0.15 (CI: 0.01–0.4) from zone A–C within 72 h (Supplementary Figure SI2). In the experiments with glass fibres, likely due to the absence of *P. ultimum* competing for the dNB nutrients, significant growth of the LP6a cells and poor predation effects on LP6a abundances were observed (Supplementary Figure SI3).

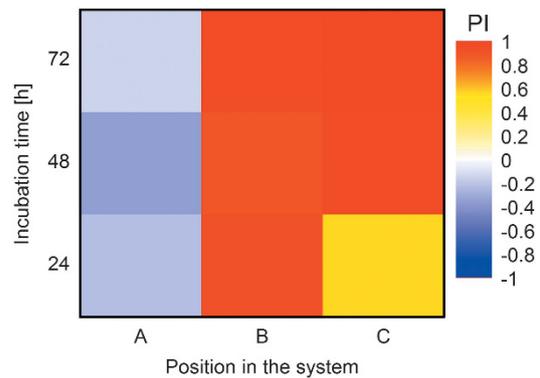


Figure 3 Effects of mycelia on the mean spatio-temporal predation impact (PI, cf. equation 1; $n=3$) by *Bdellovibrio bacteriovorus* 109J on *Pseudomonas fluorescens* LP6a. The value of PI varies between 1 (complete extinction) and 0 (no effect of predation). In case of an improved prey growth under predation pressure, the value of PI becomes negative.

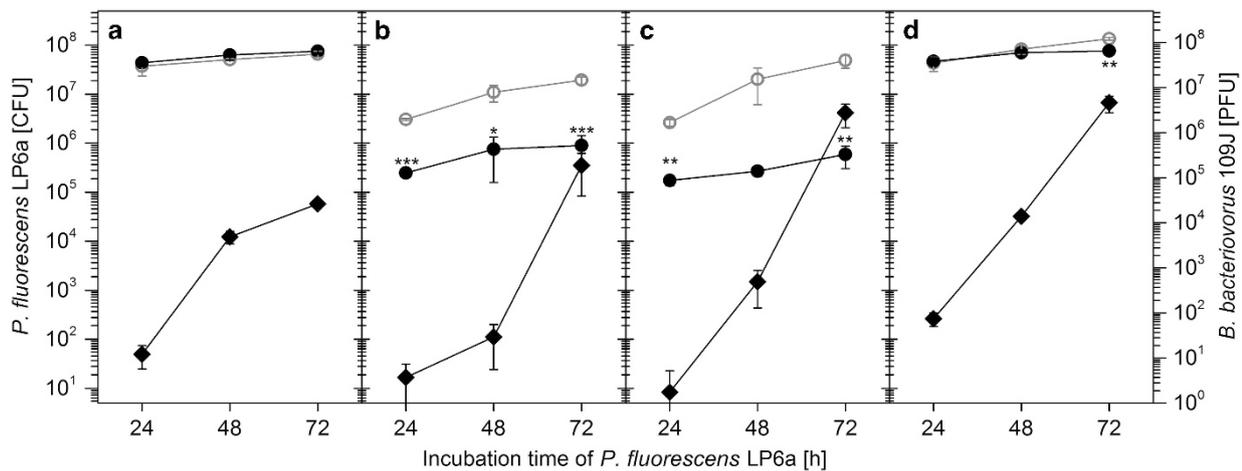


Figure 2 Population sizes of *Pseudomonas fluorescens* LP6a (colony-forming units) over time in zones (a–c) and in the overall setup (d) in the presence (filled circles) and absence (open circles) of predation pressure exerted by *Bdellovibrio bacteriovorus* 109J. Data represent averages and s.d. of triplicate experiments. Abundances of the predator as quantified by PFU are reflected by rhomboids. The mean measured colony-forming units at a distinct zone showed a statistically significant difference from the system without the predator. The asterisks refer to the statistical significance: * $P=0.05$; ** $P=0.01$; *** $P=0.001$.

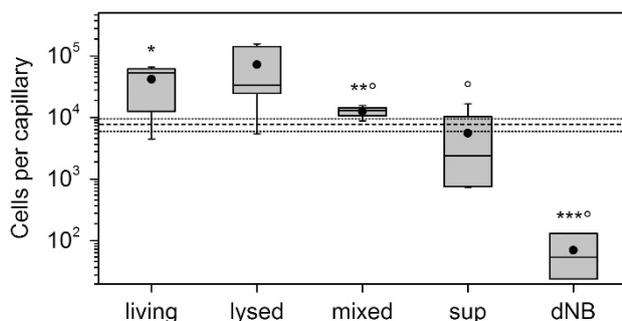


Figure 4 Number of PFU of *Bdellovibrio bacteriovorus* 109J cells in chemotaxis capillaries filled with *Pseudomonas fluorescens* LP6a ('living'), 1:1 mixtures of living and dead LP6a prey cells ('mixed'), the supernatant LP6a cells in the late exponential growth phase ('sup') and the 1:10 dNB ('dNB'). The horizontal solid and dashed lines represent the average and s.d. of the number of *B. bacteriovorus* 109J cells in capillaries filled with HM buffers only. Number of inoculated predator cells within the pond was 4×10^7 PFU per capillary. The plot shows data from six capillaries (except for data marked by the superscript ° where $n=4$ capillaries were analyzed). The asterisks refer to the statistically significant difference from control capillaries filled with HM buffer: * $P=0.05$; ** $P=0.01$; *** $P=0.001$.

Effect of *P. fluorescens* LP6a on directed dispersal of *B. bacteriovorus* 109J

The possibility of directed movement of *B. bacteriovorus* 109J to prey cells was tested in a modified Adler capillary test (Supplementary Figure SI1) using either living LP6a cells, lysed LP6a cells, a 1:1 mixture of living and dead LP6a cells, a supernatant of LP6a cells, as well as either 1:10 dNB (control) or HM buffer (control) as possible attractants. The capillary tests suggested directed movement of the predators towards the LP6a cells (Figure 4) as statistically significant 5.7-fold (CI: 3.3–8.8) and 596-fold (CI: 251–4754) more predator cells were detected in capillaries filled with living cells than in a cell-free HM buffer and the 1:10 dNB, respectively (Figure 4). Increased attraction was also observed in the presence of a 1:1 mixture of living and dead LP6a cells (HM buffer: 1.7-fold (CI: 1.3–2.3); 1:10 dNB: 180-fold (CI: 88– 2.5×10^3). Interestingly, suspensions of lysed LP6a cells showed no statistically significant accumulation of the predator either as compared with the HM buffer or 1:10 dNB (Figure 4). Avoidance of the predator was observed relative to HM buffer when capillaries were filled with 1:10 dNB or the supernatant of LP6a cell cultures, respectively (Figure 4).

Discussion

Effect of mycelia on predator dispersal and prey population dynamics

Mycelia have been found to facilitate dispersal of bacteria thereby improving ecological functions such as biodegradation and nutrient cycling (Wick et al., 2007a; Banitz et al., 2011a, b). Through their general effect on bacterial mobility, mycelia will also increase the probability of encounters between

antagonistic bacteria such as predators and prey. We thus tested the hypothesis that the highly motile bacterial predator *B. bacteriovorus* 109J is able to use mycelia for dispersal with consequences for its predation success and the population dynamics of its prey, respectively. *B. bacteriovorus* 109J was chosen for the experiments because of its ubiquitous presence in the environment and its ability to attack a wide range of Gram-negative bacteria including *Pseudomonads*, which have key functions in soil including biodegradation of organic pollutants (Abbasnezhad et al., 2011; Adebunsi et al., 2012). Pre-grown mycelia of *P. ultimum* were used to minimise theoretically possible passive spreading of predators attached to growing hyphae. Surprisingly, no dispersal of the predator along mycelia (and glass fibres as abiotic model dispersal networks) was observed in the absence of prey cells (controls III and V). As at least random dispersal of the predator along mycelia (and glass fibres) would be expected, this observation may be explained by the detection limit of our approach: given an inoculum of 8×10^3 *B. bacteriovorus* 109J cells and assuming that the predator is able to move along mycelia only (that is, not on the agar surface; cf. Supplementary Figure SI4; control VI), a one-dimensional diffusion coefficient of $D_{\text{eff}} = 5.8 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ along hyphae (Kohlmeier et al., 2005), and an effective hyphae coverage of 10% of the total area, one would calculate that approximately six predators would disperse over 1 cm to zone A within 24 h (Lawler and Limic, 2010). In presence of prey cells, by contrast, dispersal and growth of *B. bacteriovorus* 109J along hyphae of *P. ultimum* was manifested by the presence of clearly detectable numbers of predator cells in habitats accessible by the 'fungal highway' after 24 h (zones A–C). This reflects the importance of the presence of prey cells as carbon and energy sources for efficient long-distance colonisation of the mycelia by the predator. The predator's presence also clearly reduced prey populations (Figure 2). Thereby most significant effects on prey populations were observed in the least colonised zones B and C, where the relative predator impact rose along with drastically decreasing numbers of prey cells (Figure 2 and Supplementary Figure SI2) within the first 24 h after predator inoculation. Predation effects are in good agreement with previous studies on *B. bacteriovorus* predation on planktonic (Liu et al., 2014) and biofilm bacteria (Markelova and Colwell, 1999; Kadouri and O'Toole, 2005). Between 24 h and 72 h increased growth of the LP6a cells was observed in presence of the predator (zone B: $\approx 0.03 \text{ h}^{-1}$; zone C: $\approx 0.02 \text{ h}^{-1}$) as compared with its absence (zone B: $\approx 0.04 \text{ h}^{-1}$; zone C: $\approx 0.06 \text{ h}^{-1}$). No quantitative predation effect on LP6a cells was seen in the densely colonised zone A. Assuming that six to eight predator cells would be produced by one prey cell (Sockett, 2009) $\sim 8 \times 10^5$ prey cells would have been killed in order to allow for growth of 5×10^5 *B. bacteriovorus* 190J cells in zone A and,

hence, would be in the range of the s.d. of our replicate data. More interestingly, the high cell density in zone A did not inhibit dispersal of *B. bacteriovorus* 109J to zones B and C (Joubert *et al.*, 2006; McDougald *et al.*, 2012). Clear foraging effects by *B. bacteriovorus* 109J, however, became evident in the less populated zones B and C (Figures 2 and 3), suggesting that predation may be influenced by the density and the distribution of the prey. To corroborate the role of mycelia as physical dispersal infrastructures, we also installed parallel control experiments using glass fibres as artificial dispersal networks, whereby similar positive effects on the dispersal of predator cells were observed (Supplementary Figure S13). However, due to the absence of competition for substrates between prey bacteria and *P. ultimum*, high prey cell numbers and, hence, poor apparent predation effects were observed.

Previous studies have shown that *B. bacteriovorus* responds tactically to yeast extract (Straley and Conti, 1974), amino acids (Lamarre *et al.*, 1977), acetate or propionate (Straley *et al.*, 1979; Rendulic *et al.*, 2004; Chauhan and Williams, 2006) and potentially also towards its prey. We hence further tested in a modified capillary test whether dispersal of the *B. bacteriovorus* 109J would be directed by the presence of LP6a cells. Data from capillary experiments containing living LP6a cells pointed at a directed movement of the predators towards prey bacteria as evidenced by six and 596 times higher cell numbers in the capillaries filled with living LP6a cells as compared with capillaries filled with HM buffer and 1:10 dNB as used in the dispersal experiments, respectively (Figure 4). Although the effect on directed dispersal may appear small, it is statistically significant and also reproducible with a 1:1 mixtures of dead and living LP6a cells or *E.coli* ML-35 cells (*ca.* five-fold increase relative to HM buffer *cf.* Supplementary Figure S15). It is also in good agreement with an earlier study assessing targeted predator–*E.coli* interactions in bioluminescent assays (Lambert *et al.*, 2003). No directed dispersal towards the supernatant of prey cells was observed thus underpinning earlier studies (Straley and Conti, 1974). It rather seems that for presently unknown reasons intact bacteria effectively triggered directed movement, whereas no or unclear movement towards supernatants of prey cultures or cell lysates was found. A possible explanation still awaiting experimental verification would be that initial infection events are needed to trigger the attraction of further predators (Wadhams and Armitage, 2004). Data from our capillary experiments, hence, suggest that LP6a cells may act as attractant for predatory dispersal along mycelia, although this could not be underpinned unequivocally in our mycelia-based experiments.

Ecological relevance of predation along dispersal networks

Our data give evidence that hyphae increase the accessibility of prey bacteria to *B. bacteriovorus* 109J

and, hence, allow for efficient foraging and shaping of prey populations not seen in the absence of mycelia. The mere existence of mycelia, however, seems not sufficient to enable efficient long-distance migration of the predator; likely due to the absence of the prey as carbon and energy source or its role as attractant for the predator, respectively. To test our hypothesis well-controlled, tailored microbial landscapes were used for better reduction of emerging properties in complex systems. Due to the novelty of our hypotheses, it does not come as a surprise that little is known about the prevalence and ecological consequences of predator–mycelium–prey interactions in soil systems. Quantitative application of our results to complex natural environments has to be performed with great care due to the often high heterogeneity of environmental (for example, soil) habitats. Our findings and their dimension, however, suggest that mycelial networks in natural habitats are locations of similar interactions. This is corroborated by the fact that dispersal networks in soil and other habitats influence multifarious functions relying on bacterial mobility such as biodegradation (Wick *et al.*, 2007a; Otto *et al.*, 2016; Worrlich *et al.*, 2016), oxalate turnover (Martin *et al.*, 2012) or food spoilage (Lee *et al.*, 2014). Hence, it is most likely that BALOs would use available mycelia for their dispersal also in natural settings. It also can be speculated that this may increase the fitness (for example, by increased nutrient cycling) of both predator and prey communities and their ecosystem functions. The detailed consequences for the size and functioning of prey populations will be highly circumstantial and difficult to predict. Further studies including functional end points such as biodegradation rates would be needed to judge the overall effect of increased predator mobility (Varon and Shilo, 1981). At present, the effects of (possibly prey-directed) predation on ecological or functional services in soil still remain unclear. Our findings, however, pave the way to research into the prevalence of predator–mycelium–prey interactions, the underlying mechanisms and their influence on ecosystem functions and services.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

This project has been performed in the frame of the Helmholtz Association Research Programme ‘Terrestrial Environment’ and contributes to the research topic Chemicals in the Environment (CITE). It also was supported by the research program Chemical Active Transport (CAT) and Networking Fund through the Helmholtz Interdisciplinary Graduate School for Environmental Research (HIGRADE). We thank Jana Reichenbach, Rita Remer and Manuel Trost for skilled technical help and advice.

Author contributions

SO and LYW designed the experiments; SO and EPB performed the experiments; SO analysed the data; SO, LYW and HH wrote the manuscript and Supplementary Information. All authors revised the manuscript and Supplementary Information.

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