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METHOD

Bacteria-in-paper, a versatile platform to study bacterial ecology

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Abstract

Habitat spatial structure has a profound influence on bacterial life, yet there currently are no low-cost equipment-free laboratory techniques to reproduce the intricate structure of natural bacterial habitats. Here, we demonstrate the use of paper scaffolds to create landscapes spatially structured at the scales relevant to bacterial ecology. In paper scaffolds, planktonic bacteria migrate through liquid-filled pores, while the paper's cellulose fibres serve as anchor points for sessile colonies (biofilms). Using this novel approach, we explore bacterial colonisation dynamics in different landscape topographies and characterise the community composition of *Escherichia* coli strains undergoing centimetre-scale range expansions in habitats structured at the micrometre scale. The bacteria-in-paper platform enables quantitative assessment of bacterial community dynamics in complex environments using everyday materials.

Keywords

Bacteria, colonisation, dendritic networks, experimental tools, habitat structure, microfabrication, paper, population dynamics, range expansion, spatial structure.

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INTRODUCTION

The intricate spatial structure of habitats has a decisive influence on the populations they support. Community dynamics are shaped by myriad factors, from macroscopic (i.e. many body lengths) to mesoscopic (i.e. several body lengths) and microscopic (*c*. 1 body length) scales, dispersal, resource abundance, physical structure, and interactions between individuals sculpt the assembly and composition of communities (Levin 1992; Leibold *et al*. 2004). Furthermore, processes occurring at different scales are intricately linked as single genes in individual cells can shape patterns and processes at the ecosystem level and vice versa (Whitham *et al*. 2006; Wymore *et al*. 2011). For bacteria, this means that the smallest ecological scale at which they interact with their environment is on the order of micrometres, while environmental gradients extend over millimetres and beyond (Cordero & Datta 2016). Physical and chemical heterogeneities through space and time, and interactions between bacteria give rise to the diverse and architecturally complex bacterial communities we find in nature (Dethlefsen *et al*. 2017; Stocker 2012; Hol *et al*. 2013; Vos *et al*. 2013; Aleklett *et al*. 2017). While the composition and structure of bacterial communities inhabiting various habitats, including the human body (Costello *et al*. 2009) and soil (Raynaud & Nunan 2014), have been characterised in detail, we currently lack generally available tools to systematically study how the micro- and mesoscopic structure of habitats drives the assembly and dynamics of such communities (Widder *et al*. 2016). Many important bacterial habitats, including biological tissues and soil matrices, consist of a microscale network of connected pores and cavities through which individuals migrate, while their abundant surfaces facilitate the growth of biofilms. Traditional laboratory tools to culture bacteria, however, are not well suited to mimic such landscapes, and furthermore typically only support planktonic *or* surface-associated growth (not both simultaneously), and thus suppress the coexistence of these distinct lifestyles.

In the past decade, various microfabrication-based approaches to culture and study bacteria have emerged, enabling the study of bacterial ecology at the micrometre to millimetre scale by engineering synthetic landscapes (Rusconi *et al*. 2014). While such approaches have resulted in exciting insights regarding, for example, spatial competition between bacteria, the evolution of antibiotic resistance, microbial community assembly and biofilm growth (Balagaddé *et al*. 2005; Keymer *et al*. 2008, 2006; Connell *et al*. 2013; Drescher *et al*. 2013; Park *et al*. 2003; Wessel *et al*. 2013; Coyte *et al*. 2016; Hol *et al*. 2013, 2014; Hol & Dekker 2014; Kim *et al*. 2008; Nagy *et al*. 2018), microfabrication-based approaches to study bacterial ecology have not been adopted widely. This is largely due to the fact that the laboratory infrastructure necessary to create microfabricated landscapes is expensive, specialised and not readily available in microbiology laboratories. To overcome this barrier, we here demonstrate the use of paper scaffolds as a versatile and easy-to-use platform for studying bacterial communities in environments that are spatially structured at the relevant microscopic scales. Paper is a widely available material consisting of cellulose fibres.

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Interestingly, the characteristic length scales of paper (Derda *et al*. 2011) and many bacterial habitats (e.g. the soil matrix (Carson *et al*. 2010)) are very similar, having pores from a few to several tens of micrometres. Furthermore, paper can be easily cut, either by hand or using a laser cutter, into any two-dimensional geometry at the milli- to centimetre scale, while layers of patterned paper can be stacked to make three-dimensional geometries (Derda *et al*. 2009; Mosadegh *et al*. 2014, 2015; Truong *et al*. 2015). Paper furthermore can be creased and folded to create yet other geometries. The spatial scales at which paper is structured (microns) and can be manipulated (milli- to centimetre) correspond very well to the range of scales that are intrinsic to bacterial ecology, suggesting that paper may provide an excellent substrate for mimicking the complex structure of natural bacterial habitats.

We here demonstrate this novel use of paper through two case studies: a range expansion in a dendritic landscape, and the colonisation of an archipelago of uninhabited islands from an inhabited main land. Dendritic (or branching) networks, such as rivers or cave systems, comprise a ubiquitous class of landscapes and their connectivity has been suggested to influence community dynamics and biodiversity across many taxa (Campbell Grant *et al*. 2007). Theoretical studies indicate that a dendritic landscape connectivity strongly impacts community dynamics, generally leading to decreased local species richness and an increase in between-community diversity (i.e. local communities being less similar) when compared to a two-dimensional lattice with uniform connectivity (Carrara *et al*. 2012; Muneepeerakul *et al*. 2007). Dendritic ecosystems are also found at microscopic scales in, for example, lungs, capillary networks or soil, yet experimental studies investigating the ecological dynamics of bacteria in dendritic landscapes are scarce. Furthermore, as theoretical investigations of dendritic landscapes are often inspired by observations of macroscopic systems (e.g. river networks (Fernandes *et al*. 2004; Muneepeerakul *et al*. 2008)), it is interesting to experimentally test if similar mechanisms operate at microscopic scales. Experiments of protist communities undergoing experimentally imposed dendritic versus two-dimensional lattice dispersal regimes (Carrara *et al*. 2012), or colonising dendritic versus linear microcosms suggest that observations made in macroscopic systems (e.g. branching increases β diversity) translate to microbial systems, although the observed differences may be transient, eventually resulting in similar community compositions in dendritic and linear ecosystems (Seymour & Altermatt 2014; Seymour *et al*. 2015). In case study 1, we explore this question in the bacterial context by using the bacteria-in-paper approach to contrast a two-species range expansion unfolding in a dendritic landscape to a non-branching linear landscape.

In case study 2, we revisit island biogeography from a bacterial perspective. Biogeography theory posits that the community composition on islands depends on factors including the distance to the mainland and the size of the island, and for many taxa, a positive power-law scaling between habitat size and the number of species is clearly established. Observations from the field (Horner-Devine *et al*. 2004; Bell *et al*. 2005; Barreto *et al*. 2014) suggest that these generalities extend to the bacterial world, yet this notion has been a topic of debate (Fenchel & Finlay 2005), and has rarely been tested in the laboratory context (Fenchel & Finlay 2005), The bacteria-in-paper approach allows the experimental construction of bacterial archipelagoes and we demonstrate through a proof-of-concept study that paper scaffolds can be used to investigate the dynamics of bacteria that colonise initially uninhabited islands from an inhabited mainland.

As the approaches presented here do not require any specialised equipment nor specific training, we anticipate that it will enable bacterial ecologists to transition their model systems from unstructured test tubes and petri dishes, to the intricate microscopic world inside a sheet of paper.

MATERIALS AND METHODS

A detailed description and step-by-step protocol for users of the bacteria-in-paper approach are available as Supporting Information.

Preparation and inoculation of paper scaffolds

Landscape geometry was designed in Adobe Illustrator CS6 and cut in Whatman 1 Chr chromatography paper (0.18 mm thick) using a laser cutter (Versa Laser-Universal Laser VL-300). Cut paper was autoclaved and dried before use.

Dendritic assay

Separate overnight cultures of *E. coli* strain JEK1036 (W3110 lacYZ::GFPmut2) and strain JEK1037 (W3110 lacYZ::mRFP) were diluted 1/200 in fresh LB medium supplemented with 10 μM isopropyl β-D-1-thiogalactopyranoside (LB-IPTG), grown to mid log phase and mixed at 1:1 ratio for inoculation (premixing density measured by optical density). Paper scaffolds were submerged in LB-IPTG for 5 min, excess medium was allowed to drip from the paper and the medium saturated paper was suspended horizontally on a grille of thin peek tubing (5 mm pitch). The assembly was transferred to a chamber with saturated humidity and placed in an incubator set to 37 °C for 30 min prior to inoculation. The 1:1 mix of GFP- and RFP-labelled *E. coli* was inoculated onto the centre of the scaffold using a 1 microlitre inoculation loop giving rise to an inoculation zone of approximately 2 mm. Scaffolds were incubated for 15 h at 37°C.

Archipelago assay

Separate overnight cultures of *E. coli* strain JEK1036 (W3110 lacYZ::GFPmut2) and strain JEK1037 (W3110 lacYZ::mRFP) were diluted 1/200 in fresh M9 medium supplemented with 0.4% glucose and 10 μM IPTG and grown to mid log phase. Mid log phase cells were washed by spinning down, discarding the supernatant, and resuspending in M9-IPTG without glucose; washed cells were mixed at 1:1 ratio before inoculation. The paper scaffolds comprising the archipelago (one mainland, three islands) were positioned on a layer of parafilm on top of a cover slide. The parafilm was cut around the paper, and excess parafilm (i.e. parafilm not sandwiched between paper and glass) was removed. The slide was heated on a hotplate to briefly melt the parafilm and secure the scaffolds to the glass slide. After cooling 5 μL of a 20% glucose solution

was pipetted onto the paper islands (not the mainland) and let to dry. The 1:1 mix of GFP-and RFP-labelled *E. coli* was inoculated onto the mainland using a 1 microlitre inoculation loop. A rectangular Gene Frame adhesive (17 × 28 × 0.25 mm, Thermo Fisher Scientific) was placed around the paper scaffolds, and 125 μL of M9 medium with 10 μM IPTG (no glucose) was introduced. The archipelago was closed by placing a cover slip on the Gene Frame and incubated at 37°C for 15 h.

Confocal imaging of bacteria-in-paper

Archipelagoes were imaged without modification. Dendritic scaffolds were fixed by submerging in 4% formaldehyde in phosphate-buffered saline (PBS) for 15 min and subsequently washed three times with PBS. Scaffolds were imaged in PBS + 50% glycerol in an imaging chamber (cover slide, Gene Frame, cover slip). Imaging was performed on a Nikon A1R Confocal system controlled using NIS-Elements C software at 10x or 20x magnification.

qPCR of bacteria-in-paper

Fragments (2 mm^2) were cut from the extremities of the branching and non-branching landscapes using a razor blade and used for genomic DNA extraction (NucleoSpin Tissue kit, Macherey-Nagel). Quantitative PCR was performed in duplicate on an Eco Real-Time PCR system (Illumina) using SYBR Green PCR Master Mix (ThermoFisher Scientific) and primer sets GFP: 5'-GGCACTCTTGAAAAAGTCATGCT-3' (forward), 5'-CCATGGCCAACACTTGTCACT-3' (reverse), RFP: 5'-CCCTGAAGGGCGAGATCAA-3' (forward), 5'-TG GCCATGTAGGTGGTCTTG-3' (reverse). Data were analysed in MATLAB 2016a using a custom script.

RESULTS AND DISCUSSION

To facilitate bacterial growth and motility in the paper matrix, we saturated paper with bacterial growth medium (LB broth). Confocal imaging of fluorescently labelled *Escherichia coli* demonstrated that bacteria can swim in the liquid medium that fills pores in the cellulose mesh, allowing bacterial growth, migration and colonisation through paper scaffolds several centimetres in length. Figure 1 shows sessile colonies (biofilms) formed by *E. coli* (Fig. 1b and c) and *Bacillus subtilis* (Fig. 1d) after a 15 h incubation period at 37°C. Cellulose fibres act as anchor points for surface-associated growth, giving rise to dense colonies that form in the pores (see Supplementary Movie 1 for a confocal Z-stack showing bacterial aggregates that formed 0–33 μm into the paper). Before inoculation of bacteria at one central point of the paper scaffold, the entire scaffold was saturated with growth medium forming an initially homogeneous nutrient landscape. After 15 h of growth cellular aggregates had formed scattered throughout the paper scaffold – even at the extremities, centimetres away from the original inoculation point.

Confocal imaging penetrates up to *c.* 100 micrometres into the paper, enabling the high-resolution visualisation of communities of fluorescently labelled bacteria inhabiting the paper. The paper in use here weighs 87 g/m^2 and is 180 micrometres thick, images taken at multiple focal distances (Z-stacks) from both sides can thus be used to visualise the entire community. However, cellulose fibres may obscure a fraction of cells when imaging beyond several tens of micrometres into the paper. To enable quantitative assessment of the bacterial communities inhabiting the paper matrix independent of the penetration depth of imaging, we took advantage of the fact that bacterial DNA can easily be extracted from paper to assess the community composition by, for example, quantitative PCR (qPCR) or sequencing-based methods (e.g. (Cira *et al.* 2018)). As we demonstrate below, qPCR provides an economical and convenient means to spatially resolve community composition, albeit at a lower resolution compared to confocal microscopy.

Case study 1: Dendritic network connectivity

Having established that bacteria are motile and grow in paper containing growth medium, we used this approach to investigate the colonisation dynamics of *E. coil* in a range expansion in two distinct types of landscapes. To probe the effect of a dendritic landscape topology on bacterial range expansions, we cut paper scaffolds (26 × 15 mm) that consist of a central inoculation zone providing access to both a dendritic and a non-dendritic landscape on opposite sides (top and bottom in Fig. 2a respectively). As both landscapes are colonised from the same inoculation zone, and thus by the same initial community, the effect of branching on the range expansions can be assessed by comparing the community composition at the extremities of both landscapes. Paper scaffolds were saturated with rich growth medium (LB) and inoculated in the centre with a 1:1 mixture of neutrally labelled *E. coli*, isogenic except for a green fluorescent protein (GFP) versus red fluorescent protein (RFP) insertion in the *Lac* operon (Keymer *et al.* 2008; Hol *et al.* 2013; Van Vliet *et al.* 2014). A challenge to confining bacteria to liquid-saturated paper is the liquid film that forms when wet paper comes in contact with a surface (e.g. a glass coverslip). In order to prevent bacteria from growing in or migrating through such a liquid film, we suspended the wet paper on thin wires (*c.* 5 mm pitch) in a chamber with saturated humidity. This ensures that no liquid interfaces are formed and all bacterial migration happens through the paper matrix.

Confocal imaging of the branches demonstrated that bacteria successfully colonised the full length of both landscapes during a 15 h incubation period and revealed mixed (both colours) cellular assemblages at the branch extremities indicating coexistence of the two strains (Fig. 2). To determine the community composition at the ends of the range expansion, we extracted genomic DNA from 2 mm^2 paper fragments cut from the branch extremities. By utilising qPCR, we assessed the population fraction of GFP- versus RFP-labelled *E. coli* in the branches using primer pairs amplifying a fragment of the respective genes encoding for the fluorescent proteins. Quantitative PCR showed that the average (global) community composition at the branch extremities did not differ from the community composition at the far edge of the linear system (rank sum test, $P < 0.01$) nor did either average deviate

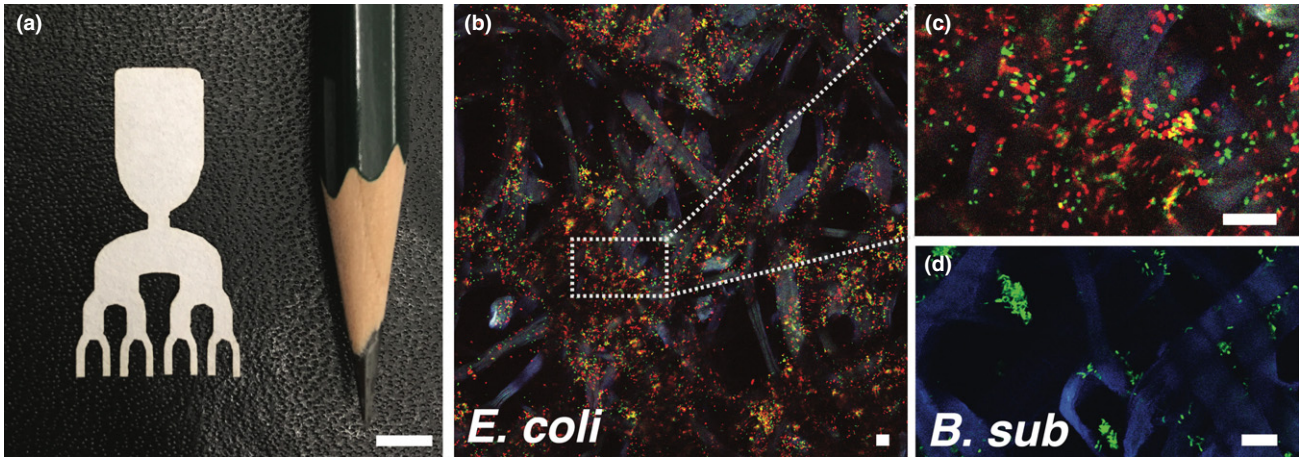

Figure 1 Bacteria-in-paper. (a) A photograph showing a paper scaffold cut to a predesigned shape with a laser cutter. A pencil is shown for scale, the scale bar is 5 mm. (b) Confocal scan of bacteria-in-paper showing *GFP*-expressing *E. coli* (green), *RFP*-expressing *E. coli* (red), and paper (blue). (c) Zoom in of the area indicated with dashed lines in (c). (d) Confocal scan of *GFP*-expressing *B. subtilis* (green) and paper (blue). Scale bars in (b–d) are 20 μm.

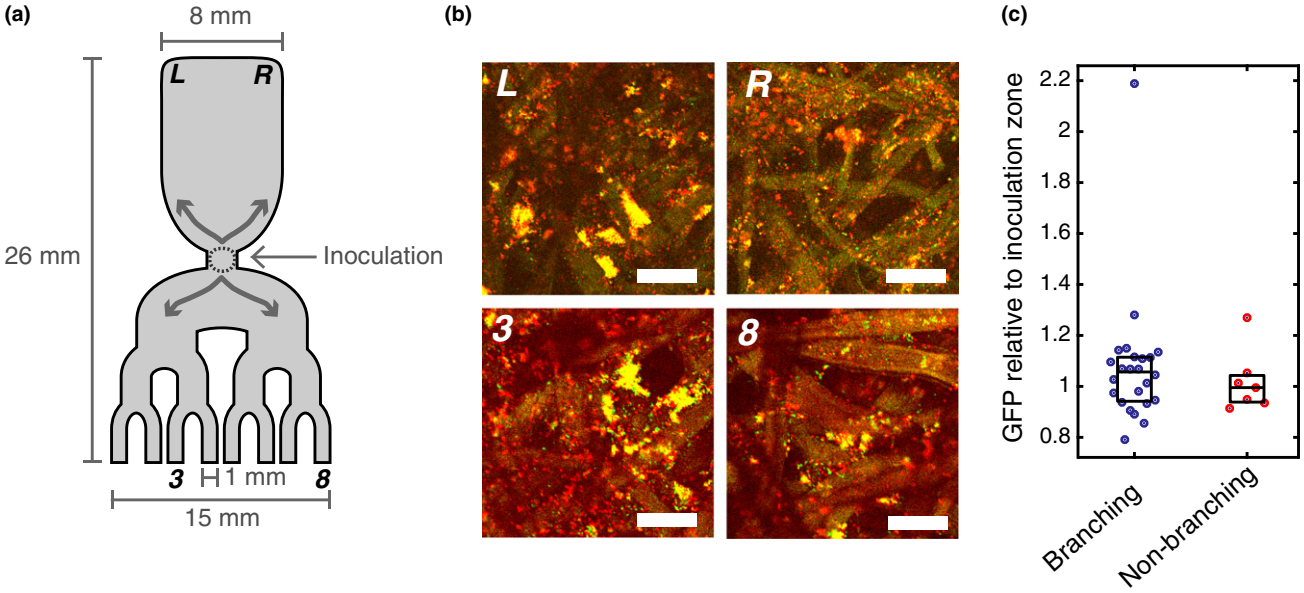

Figure 2 Range expansions in branching and non-branching landscapes. (a) Cartoon of a paper scaffold consisting of a branching landscape and a non-branching landscape connected to the same inoculation zone (indicated by a dashed circle), arrows indicate the direction of migration and population expansion upon inoculation. (b) Confocal scans of *GFP*- and *RFP*-labelled *E. coli* at the landscape's extremities labelled L, R, 3 and 8 in panel (a). Scale bars are 20 μm. (c) Fraction of *GFP*-labelled *E. coli* relative to the *GFP* fraction at the inoculation zone measured at the branch extremities by qPCR on gDNA extracted from the most distal 2 mm of each branch (i.e. all eight branches for the branching landscape, and the left- and rightmost corners of the non-branching landscape). Data are plotted for three replicate experiments ($n = 3$), the central line indicates the median, the bottom and top edges indicate the 25th and 75th percentile respectively.

from the composition of the population in the inoculation zone. Although the averages were similar, the variation in community composition between branches was larger when compared to the variation among patches of equal size in the non-branching system (one-sided *F*-test, $P = 0.03$). The observed increase in interbranch variation in the dendritic network is in agreement with theoretical predictions, yet the effect is rather modest despite the fact that the range expansion covers centimetre distances, that is, $c.10^3$ body lengths, indicating that local populations are more similar than would be expected from theory (Muneepeerakul *et al*. 2007; Carrara *et al*. 2012; Paz-Vinas & Blanchet 2015). A possible reason for this is provided by recent theoretical work showing that the distribution of the distance of dispersal events can have a profound influence on the spatial composition of populations, suggesting that a broad distribution of dispersal events (a co-occurring short- and long-distance dispersal) may increase the local diversity of populations (Paulose *et al*. 2019) decreasing the dissimilarity between local populations. The suggestion that a broad distribution of dispersal distances increases population mixing and thus positively impacts local diversity is of relevance to the experiments performed here, as bacteria

switch between two lifestyles each having a distinct dispersal mode (biofilm growth characterised by short-range dispersal only, and a planktonic form capable of much longer distance dispersal).

Interestingly, the relatively balanced population composition at the branch extremities, and the low variation between branches we observe, contrasts findings from a different experimental system commonly used to study bacterial range expansions, namely bacterial colonies growing on solid agar (Hallatschek *et al.* 2007; Hol *et al.* 2015). *E. coli* are non-motile on solid agar, and a range expansion of two neutral strains growing on solid agar starting from a mixed point inoculation is governed by a stochastic coarsening process in which a small number of pioneers quickly dominates the expanding front, diminishing local diversity (Hallatschek *et al.* 2007; Hol *et al.* 2015). The coarsening is driven by priority effects in which competition for space and resources at the colony's perimeter favours the expansion of those already present. In contrast to range expansions on solid agar, the current paper-based system supports local coexistence of the two strains throughout the range expansion. Two strain coexistence is even observed at micrometre scales within an individual branch (Fig. 2), suggesting that coarsening along the range expansion is completely absent in paper scaffolds. As alluded to above, dispersal dynamics can strongly impact community assembly (Paulose *et al.* 2019); it is therefore possible that the stark differences in colonisation dynamics in paper scaffolds compared to solid agar originate from the different modes of dispersal and growth that the two systems support: dispersal by growth and division only on solid agar, versus swimming motility and co-occurrence of sessile and planktonic lifestyles in paper scaffolds. It is interesting to note that the distinct lifestyles that the paper scaffolds support are an important ingredient of bacterial community assembly in natural habitats (Kolter & Greenberg 2006). Taken together, these results suggest that when growth and division are the only modes of dispersal, this leads to a coarsening of the community composition along the range expansion, while habitats that support the full range of dispersal modes promote community mixing to a much larger extent resulting in a high degree of diversity even at local scales.

By virtue of the presence cellulose fibres (anchors for sessile bacteria) and liquid-filled pores, paper substrates may provide a higher degree of environmental heterogeneity when compared to a solid agar surface. As spatial and environmental heterogeneity have been linked to the persistence of transient ecological dynamics (Hastings & Higgins 1994; Doebeli & Ruxton 1998; Hastings *et al.* 2018), increased environmental heterogeneity (especially in concert with long-distance dispersal) may be another factor contributing to the long-term local persistence of two-strain coexistence in paper scaffolds (which in essence may be transient) while transient dynamics are rapidly quenched on solid agar leading to local extinction of one of the two strains.

Case study 2: Bacterial island biogeography

We took advantage of the versatile nature of growing bacteria-in-paper to explore colonisation in a second, quite different ecological scenario, an archipelago of islands. We constructed a landscape consisting of a 'mainland' (used to inoculate the system) and several uninhabited islands situated

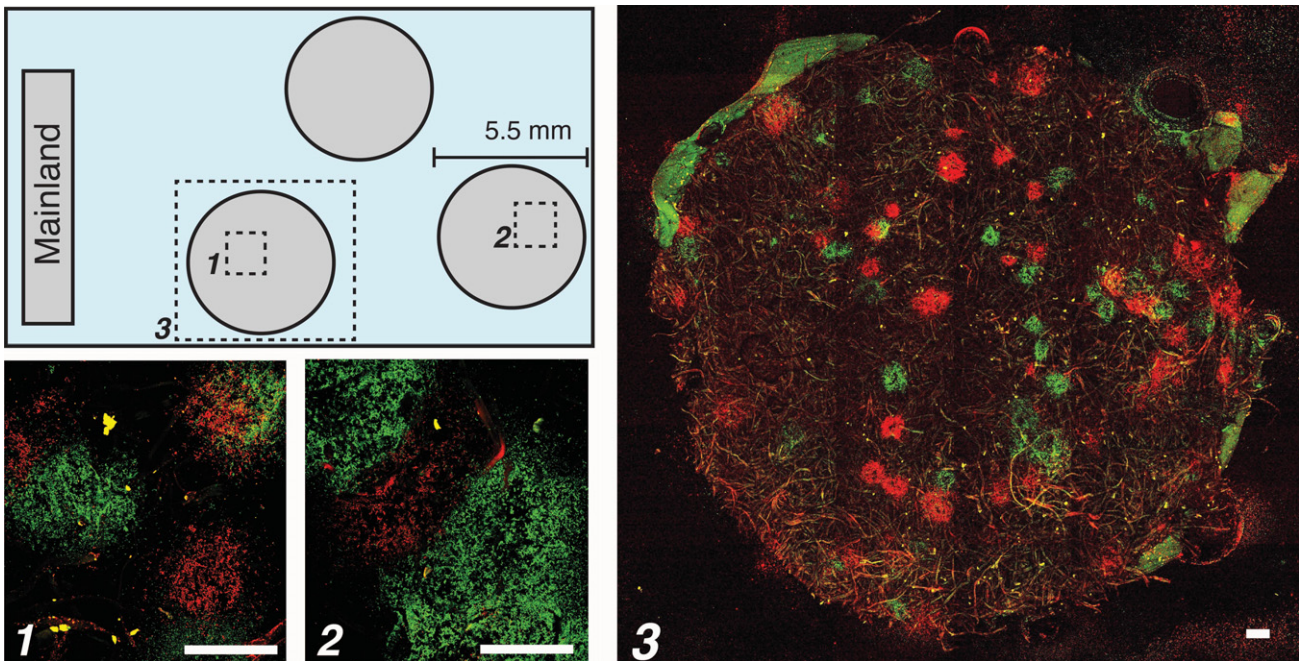

Figure 3 Colonisation of an archipelago. *E. coli* inoculated on the mainland colonise an initially uninhabited archipelago of three paper islands. Paper scaffolds are surrounded by liquid minimal medium, and (non-inoculated) islands were pretreated with glucose which starts to diffuse out of the scaffolds upon wetting. Diffusing glucose promotes bacterial migration by creating a temporary glucose gradient increasing towards the non-inoculated islands. Confocal scans correspond to the areas indicated with 1, 2 and 3 in the cartoon. Scale bars are 200 μm.

5–15 millimetres from the mainland. The (non-inoculated) islands were impregnated with 1 microgram of glucose and dried. The mainland was inoculated with a 1:1 mix of GFP- and RFP-labelled *E. coli*, the archipelago was subsequently sandwiched between glass slides and the remaining space (the 'sea') filled with minimal medium (lacking a carbon source). The bacteria thus initially faced a low-nutrient environment dotted with nutrient-rich islands. Upon wetting the landscape, the solid glucose slowly dissolved and diffused out of the non-inoculated islands, creating a dynamic and heterogeneous resource landscape. Figure 3 shows that after 15 h of incubation, *E. coli* from the mainland had successfully colonised the non-inoculated paper scaffolds and established colonies in the islands. The mainland was coloured yellow due to a uniform mix of green and red cells. Interestingly, a very different distribution of single-coloured colonies can be seen scattered across the three islands. The single-colour colonies likely derive from individual colonisers, which gave rise to distinct founder populations. Community structure at the islands may thus be driven by priority effects and differs from the mainland, exhibiting a much lower local diversity (i.e. the characteristic length scale of clonal single-colour patches is much larger) due to relatively rare colonisation events. The presence of homogeneous (i.e. single colour) regions spanning tens to several hundred micrometres suggests that the number of colonising species on small islands would be severely constrained indicating a positive scaling between island size and species richness, as predicted by classical island biogeography theory (MacArthur & Wilson 2001). As this proof-of-concept study shows, the ease with which different archipelagoes can be constructed positions the bacteria-in-paper approach well to examine the mechanisms giving rise to, for example, species–area and distance–decay relationships in bacterial communities (Green & Bohannan 2006).

Dendritic networks and archipelagos are canonical landscapes in ecology. Using no more than paper and scissors, such diverse ecological scenarios can now be explored in habitats structured at the microscopic scales relevant to bacterial ecology. Given the ease with which paper can be cut in milli- to centimetre shapes, the platform presented here can be used to address a wide range of questions on how multi-scale landscape geometry and topology affect bacterial community dynamics. In addition to spatial structure, resource heterogeneity can be incorporated by seeding nutrients locally in the paper giving rise to a rich repertoire of ecosystems that can be modelled in paper. The bacteria-in-paper platform provides excellent opportunities to investigate the mechanisms underlying the assembly of experimental (model) communities and characterising how (scaling) relations used to describe macroscopic organisms, relate to microorganisms. These aspects of microbial biogeography have been studied in field samples, yet the level of control and manipulation experimental model systems afford will provide new avenues for the quantitative testing of hypotheses posed by theoretical and modelling studies (Jessup *et al.* 2004). The approach presented here is for instance well suited to study the influence landscape topology has on the spatial scaling of microbial diversity. In addition to assessing the abundance and location of individuals/species (the 'who and where' question), high-resolution fluorescent imaging can provide a view on the physiology of a cell and the expression of relevant genes. Such measurements may provide insight into how the environment influences the physiological state of a cell, and how its state, in turn, influences its neighbours (e.g. by secreting toxins). As the state of a cell and its interactions with neighbours may have profound influences on (competitive) ecological processes, the opportunity to measure such parameters in controlled, yet realistic landscapes, presents a powerful tool for experimental bacterial ecology. Combining the bacteria-in-paper platform with next-generation sequencing will provide a means to scrutinise the genetics of local populations and explore evolutionary dynamics in spatially structured ecosystems. The evolution of cooperation in structured communities, the production of costly common goods and the emergence of resistance in spatial gradients of antibiotics are examples where the combination of bacteria-in-paper and deep sequencing could lead to novel observations.

By providing a versatile, easy-to-use and virtually zero-cost alternative to microfabrication-based approaches to experimental microbial ecology, this work fits in a broader push towards democratising science by eliminating the need for expensive and specialised equipment by providing inexpensive alternatives that rely on generic materials and tools.

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AUTHOR CONTRIBUTIONS

All authors contributed to the conceiving the study; FJHH performed and analysed all experiments, FJHH wrote the first draft of the manuscript; all authors contributed substantially to revisions.

DATA ACCESSIBILITY STATEMENT

Data available from the Figshare Repository: https://doi.org/10.6084/m9.figshare.7977245.v1

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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