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PERSPECTIVE

Build your own soil: exploring microfluidics to create microbial habitat structures

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Soil is likely the most complex ecosystem on earth. Despite the global importance and extraordinary diversity of soils, they have been notoriously challenging to study. We show how pioneering microfluidic techniques provide new ways of studying soil microbial ecology by allowing simulation and manipulation of chemical conditions and physical structures at the microscale in soil model habitats.

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Soil—a challenging habitat to study

Soil presents microbes with physical and chemical environments that vary strongly across three-dimensional (3D) space and time. Even single soil aggregates can consist of contrasting mineral materials in a matrix of air, water and organic matter, exhibiting alternating hotspots and 'desert areas' of nutrients and toxins. This complex environment challenges soil organisms that require food and shelter within the labyrinth of pores in the soil. Fragmenting barriers are likely responsible for seemingly paradoxical observations, such as the accumulation of organic material in the direct vicinity of decomposers, and make soils one of the most species-dense ecosystems in the world (Eisenhauer *et al.*, 2017; Rillig *et al.*, 2017).

Earlier approaches to address the recognized spatial and chemical complexity of soil ecosystems, such as glass bead or artificial soil model systems, and *in situ* analysis of soil with microelectrodes or tomography, have provided important insights into soil function. However, they have not been able to simulate, or manipulate, the spatio-temporal heterogeneity of soils at the microscale, the scale that microorganisms experience. Innovative microtechnologies are now emerging (Text Box 1), allowing researchers to mimic and manipulate spatially and chemically complex growth environments at the scale of soil particles (Figure 1). These approaches are opening new possibilities for addressing unanswered questions in soil microbial ecology (Table 1) by allowing researchers to construct microscopic habitats, and study interactions among individuals, distinct populations, multi-species and organism–environment interactions at the relevant scale, including at the level of single cells and hyphal tips.

In this perspectives paper, we show how microfluidics can be applied in soil microbial ecology, we present inspirational examples of microfluidic approaches in other fields such as biomedical cell research, and critically reflect on potential drawbacks and challenges to studying soil ecology with this technology.

Novel experimental possibilities using microfluidics

Microfluidic platforms have the potential to address four major challenges in studying soil systems, namely (1) their enormous spatio-temporal heterogeneity, (2) the lack of current methods to mimic soil realistically at the appropriate scale, (3) the difficulties in studying interactions among soil microbes and (4) the lack of optical access in real soil systems. We discuss these issues in turn, highlighting important advances driving the transfer of this technology to soil systems, but also identify potential challenges.

Simulating structural and chemical habitat heterogeneity

A major disadvantage of classical solid nutrient medium model systems is the absence of habitat heterogeneity. When physical barriers are absent, organisms and compounds have no constraints to

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Box 1 Microfluidics and lab-on-a-chip technology

The field of microfluidics (also known as 'lab-on-a-chip technology') allows researchers to engineer systems with precise control of fluids at the micrometer scale and thus simulate specific growth conditions (Nge *et al.*, 2013). The technique was born when microelectronic fabrication techniques were applied to miniaturize fluidic components, such as channels, valves and detectors, into a miniaturized gas chromatograph. Research continued with miniaturization of various chemical and biological analysis methods such as liquid chromatography, capillary electrophoresis, flow cytometry and DNA amplification, reducing sample volume, analysis time and equipment size. The technology was also commercially successful in ink-jet printers, flow cytometers and home pregnancy tests.

In the past decades, the biomedical field has been at the frontier of microfluidics development, miniaturizing and automating diagnostic lab methods and revolutionizing cell culturing by mimicking more realistic growth conditions. This has created a new and continuously expanding toolbox of microfluidic chip designs for biologists to study the interaction of cells and organisms with their microenvironment. For biological applications, it is common to produce microfluidic chips by molding silicone rubber (Figure 1), but also silicon and glass etching or 3D printing is often used.

dispersal. Microfluidic technology can be used to increase environmental realism by incorporating structures at the micro- to nanometer scale that confine microorganisms and prevent unintended cellular dispersal and diffusion of substrates or signaling molecules. Complex topologies can be created using two-dimensional silicone molding systems (Figures 1a–d), silicon and glass etching or 3D printing. These micro-structured chip systems provide a new platform for studying microbial interactions with their physical environment, for example, the algorithms used by fungi to explore space in search of nutrients (Held *et al.*, 2010). Conglomerations of soil particles of different sizes can be mimicked by imprinting arrays of cylinders or walls with varying diameters and shapes (Figures 1g and h), simulating soil porosity, aggregation and surface roughness (Figure 2a) (Deng *et al.*, 2015). By simulating different degrees of soil aggregation, researchers could mirror the effects of various soil cultivation practices. This new approach would allow for the analysis of soil structure effects and compaction on microbial performance, and the effect of microbes and their exudates on soil characteristics, such as moisture retention (Deng *et al.*, 2015). These new microfluidic techniques provide a great compliment to previous studies simulating soil structure through controlled assembly of different soil materials (Pronk *et al.*, 2017), different types of transparent materials (for example, glass beads, 'aquabeads' or other synthetic polymers, Downie *et al.*, 2012), and recent developments of 3D-printed soil structure proxies, designed to, for example, test how fungi explore pore spaces (Otten *et al.*, 2012).

Chemical heterogeneity, a hallmark of soil aggregates, has previously been simulated at the macroscale as patches using cut agar blocks (Boswell, 2003), in the form of gradients using tilted Petri dishes, or at the microscale by diffusion through porous materials such as ceramic plates (Wolfaardt *et al.*, 2008). While all these techniques still can be useful for simulating soil structure in experiments, they do not provide the combination of real-time optical access and precise control of physical heterogeneity, chemical conditions and liquids that microfluidics offer. A microfluidic approach to simulate chemical heterogeneity is to include a gradient generator design in the chip with stepwise mixing of fluids (Figures 1i and j), or to use diffusion gradients in hydrogels. With controlled in- and outflow of liquids, the heterogeneity is spatially stable and manipulated over time to study foraging, chemotaxis, co-existence or niche separation.

Stocker *et al.* (2008) proposed a chip design that allows for a plume-like injection of soluble compounds into the chip systems, simulating ephemeral microscale nutrient patches in marine systems. This technique could be adapted to simulate the patchy distribution of nutrients in soils at the microscale. Further, multilayer chips can combine chemical gradients with structural heterogeneity, as gradients are placed over, for example, a maze, resulting in highly controllable, spatially resolved chemostats over a heterogeneous micro-landscape.

How do microbes forage across time and space? In contrast to our understanding of macrofaunal foraging strategies, we have been constrained in identifying consistent search patterns of microbes. Pioneering work tracking *Escherichia coli* in a microfluidic maze demonstrated how bacteria self-organize into waves and how pre-stages of biofilms search for microcavities with nutrients (Park *et al.*, 2003). Similarly, using microfluidic techniques, the colonization of distinct patches and sub-micron cavities by *E. coli* and *Bacillus subtilis* have been successfully described by systematically decreasing corridor size between a densely populated patch and a nutrient-rich empty patch (Männik *et al.*, 2009). These types of experiments provide quantitative means for studying microbial dispersal across simulated soil aggregates, and could help answer fundamental questions in ecology such as the factors driving the maintenance of metapopulations by habitat heterogeneity (Keymer *et al.*, 2006).

Soil realism at the appropriate scale

The relevant spatial heterogeneity of the environment experienced by soil organisms is likely in the size range of their own cells (that is, micrometers). An environment at the microscale exhibits properties that may appear non-intuitive for us who live in a macroscale world. The surface-to-volume ratio increases with decreasing dimensions, increasing the importance of surface interactions compared to volume effects. For example, surface effects, such as

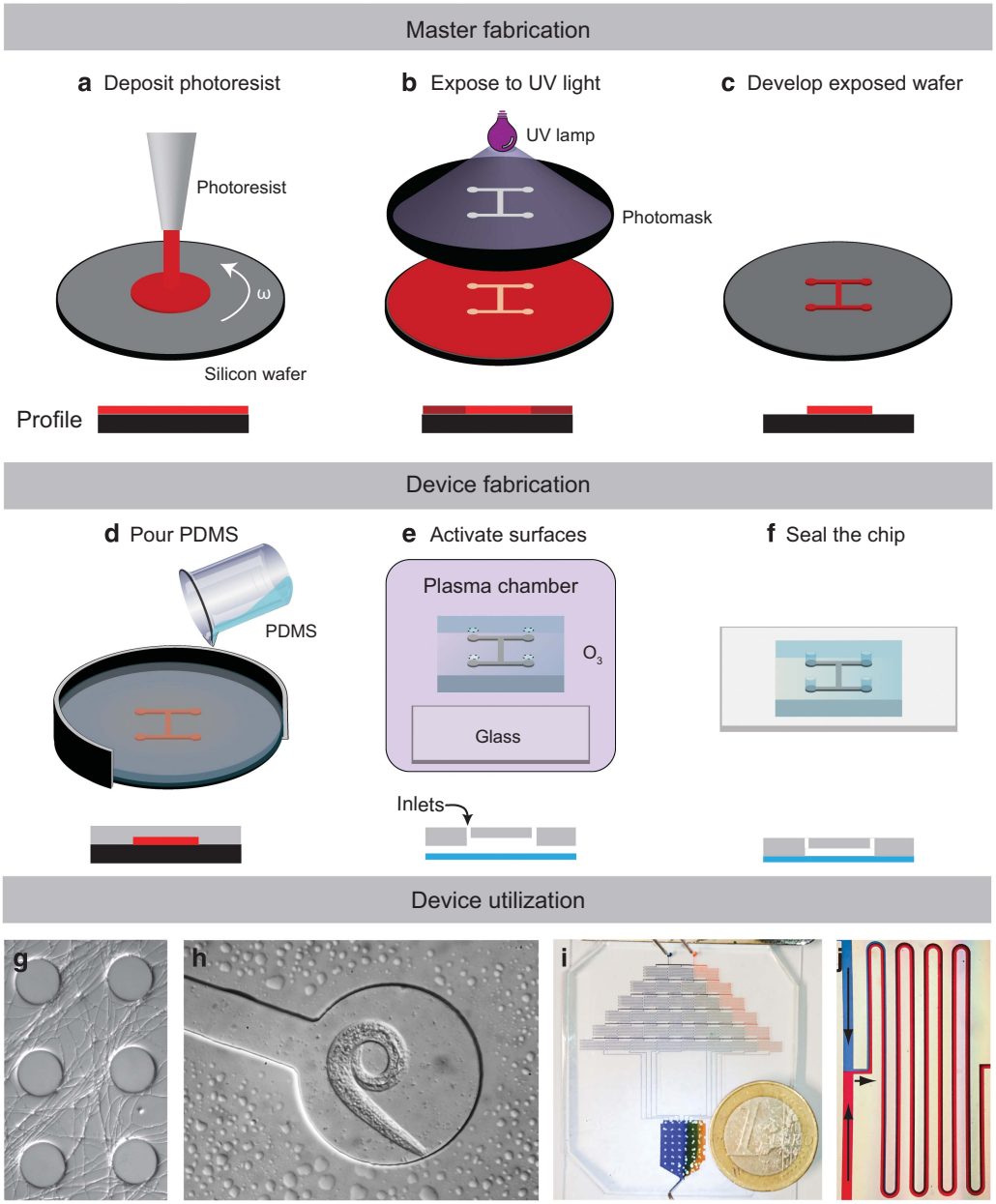

Figure 1 Fabrication of microfluidic devices. A common method to make microfluidic devices is to make a master by photolithography, which is then used to mold PDMS silicone. (**a**) Deposition of photoresist on a silicon or glass wafer. The thickness of the photoresist is defined by spinning the wafer at a certain rotational speed for a certain time. (**b**) UV light exposure through mask. UV light illuminates the desired pattern through a photomask and catalyses photoresist crosslinking. (**c**) Development of the exposed wafer. Non-crosslinked photoresist is removed using a solvent bath. The pattern is now visible on the surface of the master. (**d**) PDMS molding. PDMS is poured on the developed master and allowed to polymerize in an oven, forming a flexible polymer block. (**e**) Surface activation. Holes for desired inlets are punched into the PDMS slab, and both PDMS and the glass slide are activated in a plasma chamber. Other materials such as membranes or other PDMS layers can also be used to seal the chip. (**f**) Sealing the chip by placing the surfaces in contact, which form covalent bonds between the PDMS and the glass surface. (**g**) Hyphae of *Mycetinis scorodonius* growing in a pillar system with 100 µm wide pillars. (**h**) Nematode that migrated into a chip channel from a natural soil inoculum. (**i**) Microfluidic chip where a dye gradient is generated by sequential mixing and introduced into a culture chamber. (**j**) Zoom-in on the gradient generator showing and dye diffusion.

surface tension, capillary forces, adhesion and viscous drag, become very important at the microscale. The dominance of viscosity over inertia causes liquids to move with laminar instead of turbulent flow so that mixing mainly occurs via diffusion (Figure 1j). This means that microchannels in microfluidic systems better reflect soil conditions, where soil pore water often forms a thin film on mineral particles over which substrates can diffuse. Microfluidic systems can be inoculated with microbial isolates using 3D bacterial community printing techniques that allow precise control of the density,

Table 1 A selection of top-ranked soil and microbial ecology research questions collaboratively identified by Antwis *et al.* (2017) and Eisenhauer *et al.* (2017), and the potential benefits microfluidic approaches can provide

Research question	*Microfluidic techniques can provide*:
Do theories of macro ecology hold for microbial systems?	Possibility to incorporate microscale habitat structure into microbial model systems. Live imaging of micro-structured chambers and channels where microbial cells can compete or initiate reciprocal resource exploitation. Potential examination of niche differentiation, predator–prey relationships, food web interactions including micro-fauna, island biogeography (habitat patches within soil aggregates), the connection between microorganism biodiversity and ecosystem function, all with the aid of biosensors such as fluorescent probes.
What are the environmental triggers of microbial behavior and evolution?	Real-time visual analysis of cell interactions with complex environmental conditions. Monitoring of frequency and triggers of horizontal gene transfer. Controlled microenvironments to study quorum sensing, biofilm formation and community dynamics. Possibility to follow foraging and branching of tip-growing cells to monitor cellular decision making. Model-soil aggregates as evolutionary incubators to study microbial selection pressures. Microscale and *in situ* sampling (laser microdissection) of cells from chips to characterize gene expression or genetic networks.
How do microbes behave across short and long timescales to chemical cues?	Controlled chemical gradients or nutrient patches to study chemotaxis of single cells or hyphal tips. Gradients can be time resolved, switched on and off or being reversed. Microorganisms can be exposed to pulses of exudates or signaling molecules of interacting organisms.
What are the stages and preconditions of microbiome formation and succession?	Empirical platforms for testing stochastic vs deterministic community assembly processes. Time-lapse analysis of micro-structured incubation chambers to simulate soil aggregates and other habitats on a chip. Surface structure simulations from imprints to study microbiome formation, and successional drivers.
What cellular processes are necessary for symbiotic establishment and resource exchange between hosts and their microbes?	Microscopic channels for growing individual roots with precise control over environmental conditions and timing of symbiont exposure (for example, mycorrhizal fungi or Rhizobia). *In situ* analysis of signaling molecules. Visualization of direct cell interactions, and nutrient transport via fluorescent labels.
What specific roles do microbes play in the process of soil aggregation and organic matter stabilization?	Simulation and manipulation of microscale habitat structure to study the importance of organic matter occlusion. Injection of loose microparticles in chambers for the microbes to rearrange and aggregate. Bendable micro-pillars for microbes to physically manipulate. Mineral surface coating to study chemical interactions at microscale. Live visualization of particle aggregation process.
How can we improve and verify computer models of microbial processes, in order to upscale results to global ecosystem models?	Acquire empirical data of microbial growth, interactions and substrate usage via biosensors and image analysis. Grid-based designs for realistic and highly replicated tests of *in silico* experiments. Improved empirical base to upscale microbial processes to global models.

shape and size of the microbial communities (Connell *et al.*, 2013). Devices can further be scaled depending on the organism of interest, from bacteria (Park *et al.*, 2003) to roots (Grossmann *et al.*, 2011), to determine how structure affects microbial foraging decisions.

Facilitating microbial interactions

A major aim of soil ecology is to understand species interactions, and how these drive nutrient cycling. Microfluidic techniques offer methods for confining and directing microbes such that community composition can be controlled and specific cell-to-cell interactions can be monitored. They enable the study of exchange of metabolic compounds, signaling molecules, antibiotic resistance and interactions across kingdoms. Recent work using a device to track interactions between bacteria and fungi demonstrated how bacteria can induce phenotypic responses in fungal hyphae (Stanley *et al.*, 2014; Figure 2c). With this type of chip, the physical aspects of species interactions can be resolved such that we understand the mechanisms by which swarming soil bacteria can drive the dispersal of non-mobile fungi, and reciprocally how fungi provide physical bridges for the bacteria to cross air gaps in the soil (Ingham *et al.*, 2011). The manipulability of microfluidics allows researchers to ask questions about partner interactions and control, for example, eavesdropping at the soil–root interface to understand symbiotic communication (Figure 2e), or the ways in which predator–prey dynamics oscillate across fragmented landscapes (Hol *et al.*, 2016; Figure 2c).

The development of the iChip exemplifies a successful use of microchips to improve culturing techniques for soil microbes, and facilitates culturing of previously unculturable species. (Nichols *et al.*, 2010; Figure 2d). Physical microscale structure is the key factor to balance co-existence of the numerous bacterial species, as it prevents dominant species from overgrowing slower growing species. Combined with controlled

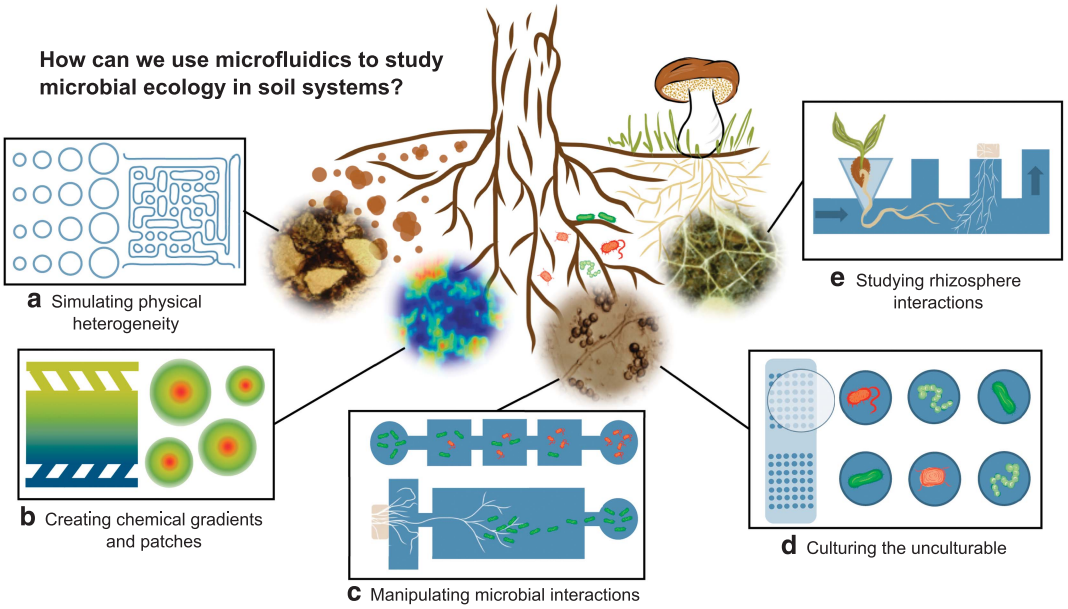

Figure 2 Five aspects of how microfluidics can be used to mimic the soil-environment and study microbial behavior in a small-structured environment. (**a**) **Simulating physical heterogeneity**. Pillars and walls of different sizes and shapes can be used to simulate differences in soil structure and porosity to study how variation in physical heterogeneity affects, for example, microbial establishment, behavior (Held *et al*., 2010; Deng *et al*., 2015), and feedback interactions with their environment. (**b**) **Creating chemical gradients and patches**. Chemical gradients or plume-like injections can be created inside the chips to mimic spatial heterogeneity of nutrients or other soluble compounds and study, for example, chemotaxis (Stocker *et al*., 2008). (**c**) **Manipulating microbial interactions**. Arenas for the study of microbial physiology, behavior and interactions can be fabricated, allowing minute control over when and where microbes enter the system, with the possibility to restrict encounters to few individual cells or hyphae (Stanley *et al*., 2014; Hol *et al*., 2016). (**d**) **Culturing the unculturable**. With the development of the Ichip (Nichols *et al*., 2010), new possibilities have opened up for culturing soil bacteria that have not previously been possible in solid medium cultures. The main design factor thought to facilitate this is the micro-confinement of individual cells in diffusion chambers sealed off with membranes, still allowing for metabolic transfer to and from the surrounding environment. This strongly expands the species pool for laboratory studies, and facilitates identification of their special requirements for pure culture isolation. (**e**) **Studying rhizosphere interactions**. Plant roots can be grown from seeds, for example, through pipette tips, into channels of microfluidic devices (Grossmann *et al*., 2011), permitting close monitoring of root morphology, and giving us the ability to control nutrient supply as well as microbial exposure within the root system. This will open up possibilities to study, for example, the colonization success and succession of root symbiosis such as those involving rhizobia and mycorrhizas, or monitoring of pathogens under differentiated nutrient conditions.

diffusion of metabolites between cavities and the surrounding environment, this structuring can provide essential food sources to neighboring species. These techniques could inspire a strategy to increase the pool of currently cultivable fungi, by allowing rarer and more nutritionally demanding species to be isolated and screened for their metabolic profiles, such as antibiotics production.

No soil-borne symbiosis has been documented in microfluidic chips yet. However, chip designs for studying initiation or symbiotic nutrient trading dynamics in rhizobia or mycorrhizal systems, or for increasing knowledge about other endophytes, may be inspired by hydroponic chip-plant cultures (Grossmann *et al*., 2011; Figure 2e). Chips can also be developed to help us better understand lichen formation and lesser-known symbiotic associations in soils, such as diverse uncultured methane-oxidizing consortia (Hays *et al*., 2015).

Optical transparency

Soils are naturally opaque. This means that imaging, even with modern microscopy, including micro-spectroscopy with synchrotron light, requires extensive sample preparation in the form of embedding and thin cutting of soil particles or the application of vacuum conditions. These procedures are known to produce artefacts such as changes in drying pore structures or chemical alterations. Recently, the development of 'transparent soils' helped to study rhizosphere activity in real time (Downie *et al*., 2012). Microfluidic devices allow for this type of analysis at an even finer scale (Grossmann *et al*., 2011), and are commonly produced in transparent materials such as glass, or transparent polymers. This facilitates both direct microscopy and extensive *in situ* analyses of microscopic samples (Nge *et al*., 2013).

Unfortunately, the most commonly used chip material polydimethylsiloxane (PDMS) adsorbs analytic radiation such as infrared light and does not allow for example, IR-, RAMAN- or scanning transmission X-ray microscopy. Nevertheless, chemical changes of substrates and metabolic activity can be made visible via fluorophores, and tracked both inside or around cells for advanced image and video analysis of soil organisms, cell growth, movement and substrate usage.

Inspiration from neighboring fields

The use of microfluidics is rapidly expanding in different fields of research. During the past decades it has been especially successful in biomedicine, where among other things it is used to mimic *in vivo* human cell culturing conditions (so called organs-on-a-chip) to study cell differentiation and tissue organization (Bhatia and Ingber, 2014). These advances can serve as important inspiration for developing microfluidic chips in soil ecology.

In neurophysiology, microfluidic systems facilitate the growth of neuronal axons under controlled conditions: chemical gradients, microgrooves and funnel-shaped micro-channels direct axonal growth to study the formation of neural networks and signaling (Millet and Gillette, 2012). Since neurons share many characteristics with other tip-growing cells, this research can serve as inspiration for the development of tools to study behavior and network formation of roots, hyphae, streptomycetes or other tip-growing organisms. The chip designs used in neuroscience are adaptable for studying similar functions within fungal networks, such as the processes of self-organization. Because of the 3D complexity of nervous tissue, multilayer microfluidic devices incorporating hydrogels have been developed. 3D micropatterning techniques have been used to control the area of cell adhesion and neurite projection by etching out collagen gel using an infrared laser beam (Odawara *et al.*, 2013). Following these examples, systems could be constructed to study soil microbial ecology in 3D space. These designs rely on multi-step chip fabrication and combinations of various techniques and materials that are technically challenging, but will be highly rewarding as pioneering techniques to follow 3D processes across heterogeneous landscapes.

Further, there is a large body of research focused on biomedically relevant cell–cell interactions, with the aim of co-culturing human cells (for example, gut or tumor cells) and bacteria (Li *et al.*, 2016). Eukaryotic cells can be placed into desired patterns with microscale resolution onto chip matrices by micropatterning of adhesive and non-adhesive agents (Millet and Gillette, 2012). Important technical problems have been solved, such as introducing cell types with contrasting requirements and growth behavior, or facilitating chemical interactions while constraining direct cell contact via membranes or nanochannels. Gut-on-a-chip designs, which aim to study human microbiomes (Li *et al.*, 2016) provide a blueprint for studies of the rhizobiome and hyphobiome studies in which thin samples of root or fungal cells can interact with bacterial colonizers. Following emerging work on plant leaf imprints (Zhang *et al.*, 2014), imprints of surface structures, such as roots and soil aggregates, can become key components in the study of microbial habitat niche separation.

Research opportunities awaiting exploration

While pioneering work has successfully exploited microfluidic techniques to study biological processes beyond medical applications, there are a number of research questions in soil ecology and general microbiology that would benefit from further development of microengineered systems. Fungi are especially understudied. Despite the importance of fungi for ecosystem services and industrial applications, we lack even the most basic understanding of their behavior at the small scale. Microsystems can help reveal how hyphal tips interact with their surroundings, identify environment-dependent foraging strategies, and study nutrient redistribution within mycelial networks. The latter may even be relevant for our understanding of self-organizing systems, as basic rules likely govern survival strategies of fungal networks.

The technical possibilities afforded by lab-on-a-chip approaches have the potential to fuel ecologists' creativity. Electrochemical sensors could facilitate the study of microbial redox processes or geoelectrical responses (Nge *et al.*, 2013), and it is also possible to separate cells of interest from complex samples based on size or mechanical properties using various microfluidic methods utilizing magnetic, electrical, optical, acoustic, mechanical, hydrodynamic or inertial forces (Lenshof and Laurell, 2010). Analytical techniques such as flow cytometry and mass spectrometry can be used to collect data from microfluidic devices to further help researchers understand the metabolic capabilities of microbial consortia, and allow us to ask larger evolutionary questions about the division of labor that emerges across communities (Hays *et al.*, 2015). Likewise, spatially resolved chemostats inside chips allow for extraction of single cells or hyphal tips from defined surroundings for genomic or transcriptomic analyses.

Challenges and limitations in future microfluidic developments

While the list of advantages and new possibilities presented by application of microfluidics to soil microbial ecology is inspiring, there are limitations and challenges that should be taken into consideration when working with microfluidic systems. Soil ecologists need to be aware that even if microfluidics enables the simulation of more true-to-life soil properties compared to solid nutrient media, the systems are still highly unnatural. Microfluidic chips are often fabricated from the silicone rubber PDMS, which is naturally hydrophobic. It can be turned hydrophilic via plasma treatment, but mimicking natural soils via controlled alterations of hydrophilic and hydrophobic surfaces is still problematic. Surface chemistry of soil minerals can be mimicked by surface coating, but high spatial resolution and

precision are challenging. Real soils, with all their physio-chemical interactions, are difficult to simulate, and while microtechnologies conveniently allow for single or a few chosen experimental factors to be individually manipulated, this reductionist approach may neglect some of the interactive effects between soil properties.

The largest current obstacle for soil ecologists to start using microfluidics may be the accessibility of the technology: While it is possible to buy commercial, ready-made chips, mainly adapted for biomedical applications (Volpatti and Yetisen, 2014), many cutting-edge questions in soil science (Antwis *et al*., 2017; Table 1), still require the development of custom designs and technical add-ons requiring interdisciplinary collaborations with engineering laboratories. However, once a specific chip design is finalized, experiments in chips can be precisely reproduced with ease, since numerous replicas of the chips can be made, and each chip can contain hundreds of internal replications of the same design features. As an alternative option, the movement of do-it-yourself biology is promoting low-tech production solutions, such as laser cutting chips in plastic materials (Walsh *et al*., 2017), that could serve research questions at the larger microscale (for example, rhizosphere studies).

While there are potential drawbacks to technology-driven science, microbial ecology is ready for the precision afforded by microtechnologies, which will complement other emerging approaches, such as tomography, NanoSIMS, STXM and 3D-printing soil structures (Otten *et al*., 2012). Microfluidics offers the ability to tackle emerging questions in soil ecology such as the hypothesis of soil aggregates as evolutionary incubators for microbes (Rillig *et al*., 2017), or phenomena such as the farming of bacteria (that is, cultivating and harvesting) by fungi when local resources become depleted in the soil (Pion *et al*., 2013) at relevant spatial scales. This has the potential to propel the field in new exciting directions (Table 1). While our ability to predict interactions in complex soils will require a range of approaches and tools, precisely manipulating conditions and inducing topological constraints on structure, nutrients and interactions will allow us to make strides in quantitatively probing soil systems.

Conflict of Interest

The authors declare no conflict of interest.

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