***Graphical abstract (for review)**

microbial consortia

environmental control

engineered

applied

bioremediation

energy

therapeutics

Better together: engineering and application of microbial symbioses

Stephanie G. Hays^{1*}, William G. Patrick^{2*}, Marika Ziesack^{1*}, Neri Oxman², Pamela A. Silver¹⁺

*first author, +corresponding author pamela_silver@hms.harvard.edu

1. Department of Systems Biology, Harvard Medical School, 200 Longwood Avenue, WAB 563, Boston, MA 02115, USA

2. MIT Media Lab, School of Architecture and Planning, Massachusetts Institute of Technology, 75 Amherst St. Cambridge, MA 02139, USA

ABSTRACT

Symbioses provide a way to surpass the limitations of individual microbes. Natural communities exemplify this in symbioses like lichens and biofilms that are robust to perturbations, an essential feature in fluctuating environments. Metabolic capabilities also expand in consortia enabling the division of labor across organisms as seen in photosynthetic and methanogenic communities. In engineered consortia, the external environment provides levers of control for microbes repurposed from nature or engineered to interact through synthetic biology. Consortia have successfully been applied to real-world problems including remediation and energy, however there are still fundamental questions to be answered. It is clear that continued study is necessary for the understanding and engineering of microbial systems that are more than the sum of their parts.

INTRODUCTION: THE BENEFITS OF LIVING TOGETHER

Communities dominate the microbial world; coexisting organisms cannot help but interact. Interactions include touching, using dedicated signals, horizontal gene transfer, ‘competitive or cooperative’ scenarios where microbes compete for or provide resources, and alteration of environment to influence the growth of neighbors.[1–6] Cultures that consist of multiple microbial species, by definition, contain an increased range of genes and metabolic capabilities in comparison to monocultures. This diversity allows for the emergence of communal properties such as robustness and division of labor. In order to engineer consortia, scientists take control of the environment and organisms through devices and synthetic biology. These tools enable the application of microbial communities to real world problems where the natural attributes of consortia are valuable.

ROBUSTNESS

Robustness - the ability to survive perturbation - is an emergent property of microbial communities and is necessary for survival in the wildly fluctuating world.[5–7] Such robustness is demonstrated in lichens and biofilms. Lichens are collections of bacterial, fungal, and algal symbionts that can survive desiccation, irradiation, and starvation better together than isolated organisms (Figure 1a).[8,9] Biofilms, microbial communities that adhere to surfaces, exhibit similar community resilience.[10,11] Biofilms are a dominant form of bacterial growth and impact human health by coating our bodies and medical devices, contributing to biofouling, virulence, and pathogenesis. In a model of ear infections, Perez *et al.* shows biofilms of *Moraxella catarrhalis* and *Streptococcus pneumoniae* are more robust to antibiotic exposure than axenic films (Figure 1b). This results from *M. catharrhalis* protecting *S. pneumoniae* from β -lactam antibiotics and *S. pneumoniae* protecting *M. catharrhalis* from macrolide drugs.[11] This steadfastness of microbial communities may be one fundamental reason that biofilms form.[12]

DIVISION OF LABOR

Consortia have access to expanded functional and metabolic capacities allowing for division of labor across organisms.[5,6] This is clearly seen in *Chlorochromatium aggregatum*, a motile photosynthetic community in which symbionts live on the surface of their partner, a central β -proteobacterium, as epibionts (Figure 1c).[13] The green sulfur bacterial epibionts hitch a ride on the β -proteobacterium for chemotaxis and scotophobotaxis (movement away from the dark).[13] The non-photosynthetic β -proteobacterium gain access to energy from the epibionts.[14] Liu *et*

al. determined that the β -proteobacterium depends largely on the photosynthetic epibionts for energy through metabolic coupling and, potentially, electron transfer.[15] The β -proteobacterium has a reduced genome in comparison to free-living relatives but gains photosensitive motility despite its inability to do photosynthesis indicating increased evolutionary fitness when with its epibionts.[15]

The roles in microbial communities are not always well defined. ‘Omics approaches enable the determination of organisms present in communities and help assign functions to those microbes.[16,17] Two recent examples include Grube *et al.*’s look at lichen and Embree *et al.*’s investigation of methanogenic communities (Figure 1d,e).[18,19] Both consortia are complex: they contain many species and can be hard to tease apart as many isolated microbes are not readily culturable.[20] Grube and colleagues collected metagenomic and proteomic data for the lichen *Lobaria pulmonaria* and showed bacteria support lichen with increased nutrient access and resistance to external factors.[18] Embree *et al.* used single-cell genomics and metatranscriptomics under different growth conditions to reveal the functions and interactions of *Smithella* species, *Methanosaeta consilii*, and other consortia members during methanogenic alkane degradation.[19]

Not only are there advantages to dividing labor - there can be additive benefits from sharing labor with diverse partners. Ho and colleagues observed this in methanogenic communities composed of *Methylobomonas methanica* and various heterotrophs seeing increased methane oxidation rates correlated with heterotrophic diversity.[21] While this supports ecological theory and examples in higher organisms, experiments showing the importance of diversity have yielded varying results, highlighting the need for further study.[22,23]

ENGINEERING CONSORTIA: ENVIRONMENT CONTROL AND SYNTHETIC BIOLOGY

Building a system in an effort to understand it is common throughout science.[24] While there are many approaches taken to engineer microbial communities, environmental control – control of physical space and chemical environment – is an important tool that engineers can leverage.[25] Building communities can also be accomplished from the bottom up with the tools of synthetic biology.[24]

ENVIRONMENT CONTROL

Physical and chemical environment play critical roles in community function. Here we review several technologies that enable the exploration of various environmental parameters, such as the chemical concentration, time-scale, volume and spatial distribution, and their effects on cocultures.

A. MICROFLUIDICS

Microfluidic devices provide the means to study microbial ecology and fabricate micron-scale environments with precisely tuned conditions (Figure 2a). Cocultures have been studied using microfluidics in several ways: generating gradients (Figure 2ai),[26-29] trapping cells to study interactions (Figure 2aii),[26,29] creating microhabitats to observe dynamics in particular relationships (e.g. predator-prey, resource-competition), constructing and dispersing biofilms,[30] and high-throughput, droplet-based experiments (Figure 2aiii).[31] While these devices have the advantage of being parallelizable,[28,32] analysis is typically limited to optical measures of growth. Adapting microfluidic systems to other analytical techniques (e.g. flow

cytometry, mass spectrometry, liquid chromatography) would enable high-throughput collection of richer data.

B. MEMBRANE SEPARATION

Membranes used in coculture experiments can block the movement of cells while allowing small molecules (e.g. nutrients, gases, metabolites) to diffuse (Figure 2b). Membranes have been widely used to culture “unculturable” microbial consortia *in situ* or in simulated soil and marine environments (Figure 2biii).[20,33-35] Devices using this approach have isolated high-value microbes, such as methanotrophs from wetland and landfill environments[36] and, recently, over 10,000 soil microbes, one of which produces a novel antibiotic.[37] Clonal populations can be isolated from the environment by diluting environmental cell samples and inoculating membrane bound wells[35] or tubes,[38] such that each chamber contains one cell. Many of these microbes may survive as individuals only because they can share molecules needed for growth between wells.

Membranes can also control species position within a coculture. *Kim et al.* used membranes to alter spatial distribution and research cell-to-cell communication influencing the success of syntrophic soil consortia (Figure 2bi). The distance between the three microbes was varied from 0 to 1.8 mm and cocultures stabilized only at intermediate distances (600 - 1200 μm).[39]

C. SPATIAL PATTERNING

Spatial patterning (two and three-dimensional) has been used to explore how distribution affects microbial consortia (Figure 2c).

Molding *Moffitt et al.* molded tracks to embed a single cell into solid media (Figure 2ci), allowing the authors to optically measure growth of microbial consortia for 30-40 generations and examine how the vertical spacing of *E. coli* auxotrophs in separate linear tracks alters growth.[40]

Inkjet printing Inkjet printers can pattern picoliter-scale droplets of microbes, mammalian cells, and relevant biomolecules at high resolution ($\leq 25\mu\text{m}$, Figure 2cii).[41] Drachuk *et al.* recently demonstrated inkjet printing environmental biosensors. Two strains of *E. coli*, each modified respond to an environmental chemical, were printed on arrayed silk ionomers, which improved their functionality for up to 3 months.[42]

3D printing Multi-photon polymerization can be used to 3D print microscale geometries around bacteria (Figure 2ciii). Connell *et al.* used this method to create spatially separated communities of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The bacteria demonstrated greater resistance to β -lactam antibiotics when separated by the 3D printed gelation walls than when living together in a mixed environment.[43]

Fiber structure *Kim et al.* used a coaxial extruder to create calcium alginate fibers embedded with *Sphingobium chlorophenolicum*, a pentachlorophenol (PCP) degrader, in a core layer surrounded by *Ralstonia metallidurans*, a mercury ion reducer (Figure 2civ). In liquid coculture, *S. chlorophenolicum* suffers from mercury exposure, but in the structured fiber, *R. metallidurans* protects *S. chlorophenolicum*. The resulting coculture degrades both mercury and pentachlorophenol.[44]

SYNTHETIC BIOLOGY

Synthetic biologists strive to rationally engineer new functions into biological systems.[24] While many synthetic transcriptional, translational, and post-translational components and devices have been built in single cells, engineering entire populations is a new frontier.[5] Here we review work engineering bacterial communication and the application of these devices to generate complex synthetic population behaviors.

BUILDING COMMUNICATION

Interaction is key to building consortia. Recent approaches in engineering microbial communication have yielded sophisticated ways to control population density,[45,46] gene expression within one[47-49] or multiple species[50] as well as biofilm formation.[30,51]

Communication within cocultures can be controlled through metabolite crossfeeding (Figure 3a).[1,2,5,6] Much work has been done using auxotrophs[52-56] and mixed species systems.[57,58] Wintermute *et al.* performed a comprehensive analysis of pairwise *E. coli* auxotroph interactions identifying a subset of highly cooperative partners.[54] Mee *et al.* extended this idea by systematically testing interactions between auxotrophs in multi-member *E. coli* communities. Costly amino acids (Met, Lys, Ile, Arg and aromatics) promoted strong cooperative interactions and complex cocultures of 14 auxotrophs revealed a number of key dominant strains and syntrophic interactions. [55]

Quorum sensing (QS), a density-dependent form of communication, also serves as a means to program population interactions (Figure 3b).[1,2,5,6] Youk and Lim showed that a simple secrete-and-sense QS motif could generate a wide range of “social behaviors”. Secretion rate and receptor abundance were systematically altered while additional logic functions (e.g. feedback loops) were introduced. Single cell behaviors ranged from communication with neighbors to communication with ones self and intermediates thereof.[59] This is just one example of the flexibility of QS which can, subsequently, be engineered into more complex systems spanning different kingdoms.[60]

Recently Goni-Moreno and colleagues engineered interactions via conjugation. Conjugation is a method of communication that can transfer more information, in the form of DNA, and be more specific than either metabolic crossfeeding or QS. Using conjugation, Goni-Moreno *et al.* constructed a multicellular system of several strains acting analogously to computational networks for the biocomputation of logic functions (Figure 3c).[61] They first built a consortium that fluoresces only in the absence of two signaling molecules, a computation known as a NOR function. This concept was then successfully expanded to a three-strain system. [61]

Another way to engineer communication is to exploit toxin production. Weber and colleagues used toxin production to construct a synthetic microbial consortium to study species dispersal.[73] The model three-strain *E. coli* consortium consisted of: (i) a strain capable of toxic colicin production, (ii) a colicin-sensitive strain, and (iii) a resistant strain (Figure 3d). Strain growth rates were tuned via genetic engineering and the system was modeled computationally. Consortia exhibited robust proliferation with balanced growth rates when low numbers of toxin producers were included at inoculation or toxin range was short.[62] A major challenge for ecological research is to understand how competing species survive in rapidly changing

environments and here a synthetic consortium elucidated basic principles that may help to explain more complex ecosystems. [62-64]

APPLYING CONSORTIA

There are many complex tasks that consortia are well suited to address. In this review we emphasize applications in bioremediation and bioenergy. However, important work with microbial communities has also been done in wastewater treatment, nitrogen fixation in soil, and other fields.

In bioremediation, which repurposes or engineers organisms to clean up pollutants, division of labor is crucial because complex toxic compounds often require several steps for degradation and cultures must be robust to whatever compounds are present making microbial communities an ideal solution.[65-71] Petroleum can be degraded naturally by consortia that produce biosurfactants which make hydrocarbons accessible through emulsification.[69] Mnif *et al.* successfully mimicked this concept synthetically by pairing a three-microbe degradation consortium with surfactant producing strains of *Bacillus subtilis* and *Acinetobacter radioresistens*. Diesel biodegradation was more successful in cocultures with surfactant producers than in cultures of degraders alone (Figure 4a).[72]

Movement towards a greener economy will be aided by consortia, notably in the areas of renewable feedstocks, bioproduction, and biofuels. For example, consider the growth of oleaginous microalgae for the production of biofuels. Do Nascimento *et al.* showed that culturing the microalgae *Ankistrodesmus*, with *Rhizobium* increased growth as well as lipid content in cocultures in comparison to axenic cultures.[73] Similar benefits from communities have been seen in efforts to use cellulosic feedstocks for energy, which is a costly metabolically but is effectively achieved in consortia.[74,75] Cocultures have been engineered to take the system one step further by going from cellulose to product.[76-78] Minty and colleagues accomplished this by pairing a fungus that breaks down cellulose with engineered *E. coli* that make isobutanol (Figure 4b).[79] This is an excellent example of marrying natural and synthetic systems as the fungus, *Trichoderma reesei*, can degrade feedstocks without modification while the *E. coli* was engineered and optimized for isobutanol production.[79]

Microbial fuel cells (MFCs) are another bioenergy application for microbial consortia. These systems can treat wastewater and simultaneously generate energy. In MFCs, microbes degrade complex compounds in wastewater into substrates for “exoelectrogens”.[81] These electrochemically active bacteria transfer electrons onto an anode via direct contact, secreted mediators or nanowires.[81] In order to gain better insight into interactions in MFC consortia, Bourdakos *et al.* constructed a synthetic consortium consisting of *E. coli* and *Geobacter sulfurreducens*. They found that *E. coli* scavenge oxygen that is toxic to *G. sulfurreducens* enabling MFC operation in aerobic conditions. However, production of succinate by *E. coli* reduced the efficiency of the fuel cell. It would be desirable to include a species that generates less succinate in this synthetic system.[82]

CONCLUSIONS

Most natural microbes exist in consortia that provide robustness and broad metabolic capacities and these traits are attractive for applications in energy, environment, and potentially healthcare.

For example, microbial communities could be living therapeutics with health-promoting functions. Microbes have been engineered to sense and kill pathogens [83-85] however integration of engineered bacteria into the gut microbiota, a complex microbial community, is key.[86-88] To this end, Kotula and colleagues engineered bacteria that survive in the gut to detect signals that generate memory assessable in fecal samples.[86] Next steps for microbial consortia in healthcare could include combining *in vitro* sensing, killing, and nutrition augmentation systems with *in vivo* integration strategies to generate living therapeutics (Figure 4c) that can compete with natural microbiota, disperse pathogens, and foster healthy human microbiomes.

Advances in both environment engineering and synthetic biology have enabled engineering of “simple” microbial consortia. The challenges faced constructing these rather basic consortia reveal the vast complexity of microbial interactions. We believe that to realize the full potential of consortia-based biotechnological applications ‘omics scale data paired with computational models are needed to inform the selection of organisms and genetic parts and understand these systems. Much progress has been made in these fields and each is worthy of a devoted review. In future work, we hope the scientific community will engineer understandable, controllable consortia of higher complexity with increased robustness and longevity. For that goal further understanding of natural consortia and advances in both device and genetic engineering will be vital.

ACKNOWLEDGEMENTS

We would like to thank the members of our labs, especially David Riglar, Tyler Ford, and John Oliver, for their insightful comments on this manuscript as well as our support from the National Science Foundation DGE1144152, Department of Energy DE-SC0012658 “Systems Biology of Autotrophic-Heterotrophic Symbionts for Bioenergy”, SynBERC, the MIT Media Lab, and the Wyss Institute for Biologically Inspired Engineering.

FIGURE 1

Figure 1. Emergent properties in natural consortia. a. The isolated fungal (red) and algal (green) symbionts of lichen, like the *Cladonia* species pictured,[89] are less robust to harsh conditions than the complete lichen (red fungi and green algae together).[8,9] b. Biofilms that can cause inner ear infections[90] made up of *M. catarrhalis* or *S. pneumonia* are sensitive to (dashed lines) β -lactam and macrolide antibiotics, respectively but can survive (solid coloration) both antibiotics when in coculture.[11] c. Division of labor is accomplished in *C. aggregatum* with the green sulfur photosynthesizing and the β -proteobacterium providing light-sensitive motility.[91,13-15] d. Bacterial symbionts have been shown to contribute multiple processes the lichen *Lobaria pulmonaria*. [92,18] e. Potential roles of different organisms in methanogenic communities were determined by Embree *et al* demonstrating division of labor within the community.[93,19]

FIGURE 2

Figure 2. Engineering environment. a. Microfluidics are used to create specific environments, miniaturize and parallelize coculture chambers. These systems include (i) cell density and chemical gradient generators,[26,30] (ii) cell traps designed to capture single cells of two different species within a confined area,[26] (iii) and droplet generators that encapsulate single cells of multiple species in a small droplet.[94] b. Membranes can separate species. They are used in (i) transwell membranes to spatially separate cells [39] and (ii) in flow devices. [32] Membranes are also used to culture “unculturable” species *in situ* by separating single cells from samples within a device that is then submerged into an environment.[33,34,36] c. Techniques are used to pattern cells in 2 and 3D, including (i) molding single-cell wide tracks in agarose,[40] (ii) inkjet printing cells,[41] (iii) 3D printing microenvironments using multiphoton 3D printing of gelatin,[43] and (iv) creating coaxial calcium-alginate fibers with one species in the core and another in the exterior.[44]

FIGURE 3

Figure 3. Engineering microbial communication. a. Metabolic crossfeeding. One strain of *E. coli* overproduces metabolite A (blue oval) but cannot produce metabolite B (red hexagon). The second strain cannot produce metabolite A but overproduces B. Neither strain can survive in isolation but they can survive as part of a community.[49,50] b. Quorum sensing.[1,2,5,6] Cells produce a diffusible molecule (autoinducer, red dot) at a constant rate. At low cell density, the concentration of autoinducer remains low and the cell maintains an “OFF” state. At high cell densities, local autoinducer concentration increases and a responsive promoter (blue arrow) is switched ON resulting in expression changes (green hexagon).[54] c. Conjugation. The sender strain contains a plasmid (red circle) encoding a transcription factor (TF, grey oval). Upon conjugation into the recipient TF induces GFP expression. In the presence of signal A or B TF is recombined out of the plasmid. Then when the plasmid is conjugated it no longer induces transcription.[56] d. Toxins. The consortium consists of three strains: strain 1 (yellow) produces a toxin, strain 2 (red) is resistant to that toxin but overexpresses a certain molecule that constitutes a metabolic burden and strain 3 (green) is sensitive to the toxin but does not contain a burden.[57] This system is called a “Rock-Paper-Scissors” scenario and can be used to study ecological phenomena.

FIGURE 4

Figure 4. Applied microbial consortia. a. Bioremediation of petroleum contaminants by degrader consortia (grey) show that the addition of surfactant makes hydrocarbons more available to degradation and cocultures with *B. subtilis* (red) and *A. radioresistans* (purple) producing biosurfactants enable better contaminant degradation than the degrader strains alone.[72] b. Bioenergy from complex feedstocks. The fungi *Trichoderma reesei* (green) secretes cellulase which degrades weed lignocellulose into accessible saccharides (black hexagons). An engineered *Escherichia coli* strain (yellow) converts these sugars into fuel compounds.[79] c. Living therapeutics. An engineered bacteria (yellow) containing a trigger and a memory device were fed to mice and reported an event in the gut with a colorimetric output (blue).[86] Moving forward to engineer living therapeutics, it is important to note that engineered cells (yellow) must i) survive in the gut ii) integrate into the complex gut microbiota (grey), iii) sense their environment (purple hexagons), iv) respond to the environment (e.g. changing gene expression (blue coloration), providing something helpful (green squares), killing pathogens), and V) report the state of the gut after excretion.[3,86]

REFERENCES

1. Wintermute EH, Silver PA: **Dynamics in the mixed microbial concourse.** *Genes Dev.* 2010, **24**:2603–14.
 2. Song H, Ding M-Z, Jia X-Q, Ma Q, Yuan Y-J: **Synthetic microbial consortia: from systematic analysis to construction and applications.** *Chem. Soc. Rev. Chem. Soc. Rev* 2014, **6954**:6954–6981.
- * We would be remiss to not direct the reader to this comprehensive review about synthetic microbial consortia. It provides an excellent overview about the development of engineering principles and provides compelling examples for applied microbial consortia.
3. Yaung SJ, Church GM, Wang HH: **Recent progress in engineering human-associated microbiomes.** *Methods Mol. Biol.* 2014, **1151**:3–25.
 4. De Roy K, Marzorati M, Van den Abbeele P, Van de Wiele T, Boon N: **Synthetic microbial ecosystems: an exciting tool to understand and apply microbial communities.** *Environ. Microbiol.* 2014, **16**:1472–81.
 5. Brenner K, You L, Arnold FH: **Engineering microbial consortia: a new frontier in synthetic biology.** *Trends Biotechnol.* 2008, **26**:483–489.
 6. Mee MT, Wang HH: **Engineering ecosystems and synthetic ecologies.** *Mol. Biosyst.* 2012, **8**:2470–83.
 7. Stenuit B, Agathos SN: **Deciphering microbial community robustness through synthetic ecology and molecular systems synecology.** *Curr. Opin. Biotechnol.* 2015, **33**:305–317.
 8. Zhang T, Wei J: **Survival analyses of symbionts isolated from *Endocarpon pusillum* Hedwig to desiccation and starvation stress.** *Sci. China. Life Sci.* 2011, **54**:480–9.
 9. Kranner I, Cram WJ, Zorn M, Wornik S, Yoshimura I, Stabentheiner E, Pfeifhofer HW: **Antioxidants and photoprotection in a lichen as compared with its isolated symbiotic partners.** *Proc. Natl. Acad. Sci. U. S. A.* 2005, **102**:3141–6.
 10. Lee KWK, Periasamy S, Mukherjee M, Xie C, Kjelleberg S, Rice S a: **Biofilm development and enhanced stress resistance of a model, mixed-species community biofilm.** *ISME J.* 2014, doi:10.1038/ismej.2013.194.
 11. Perez AC, Pang B, King LB, Tan L, Murrah KA, Reimche JL, Wren JT, Richardson SH, Ghandi U, Swords WE: **Residence of *Streptococcus pneumoniae* and *Moraxella catarrhalis* within polymicrobial biofilm promotes antibiotic resistance and bacterial persistence in vivo.** *Pathog. Dis.* 2014, **70**:280–8.

12. Wolcott R, Costerton JW, Raoult D, Cutler SJ: **The polymicrobial nature of biofilm infection.** *Clin. Microbiol. Infect.* 2013, **19**:107–12.
 13. Frostl J, Overmann J: **Physiology and tactic response of the phototrophic consortium “Chlorochromatium aggregatum”** *Arch. Microbiol.* 1998, **169**:129–35.
 14. Cerqueda-García D, Martínez-Castilla LP, Falcón LI, Delaye L: **Metabolic analysis of Chlorobium chlorochromatii CaD3 reveals clues of the symbiosis in “Chlorochromatium aggregatum”.** *ISME J.* 2014, **8**:991–8.
- * This work takes a look at the interesting microbial community known as *Chlorochromatium aggregatum* and utilizes previous results at the 'omics level. The group completes a reconstruction of metabolism and did flux analysis to lend further insight into this symbiotic interaction. This reference is well paired with the following Liu *et al.*
15. Liu Z, Müller J, Li T, Alvey RM, Vogl K, Frigaard N-U, Rockwell NC, Boyd ES, Tomsho LP, Schuster SC, et al.: **Genomic analysis reveals key aspects of prokaryotic symbiosis in the phototrophic consortium “Chlorochromatium aggregatum”.** *Genome Biol.* 2013, **14**:R127.
 16. Krause S, Le Roux X, Niklaus PA, Van Bodegom PM, Lennon JT, Bertilsson S, Grossart H-P, Philippot L, Bodelier PLE: **Trait-based approaches for understanding microbial biodiversity and ecosystem functioning.** *Front. Microbiol.* 2014, **5**:251.
 17. Zengler K, Palsson BO: **A road map for the development of community systems (CoSy) biology.** *Nat. Rev. Microbiol.* 2012, **10**:366–72.
 18. Grube M, Cernava T, Soh J, Fuchs S, Aschenbrenner I, Lassek C, Wegner U, Becher D, Riedel K, Sensen CW, et al.: **Exploring functional contexts of symbiotic sustain within lichen-associated bacteria by comparative omics.** *ISME J.* 2014, doi:10.1038/ismej.2014.138.
- ** Even when deconstructing lichens, we often think of algae and photobionts overlooking the important role heterotrophic bacterial symbionts play. This is an interesting paper that addresses that aspect of lichen symbiosis while clearly demonstrating division of labor across species.
19. Embree M, Nagarajan H, Movahedi N, Chitsaz H, Zengler K: **Single-cell genome and metatranscriptome sequencing reveal metabolic interactions of an alkane-degrading methanogenic community.** *ISME J.* 2014, doi:10.1038/ismej.2013.187.
 20. Stewart EJ: **Growing unculturable bacteria.** *J. Bacteriol.* 2012, **194**:4151–4160.
 21. Ho A, de Roy K, Thas O, De Neve J, Hoefman S, Vandamme P, Heylen K, Boon N: **The more, the merrier: heterotroph richness stimulates methanotrophic activity.** *ISME J.* 2014, **8**:1945–8.

*Biodiversity and its effect on function is a difficult topic to address. This is one simple example where biodiversity is shown to be very important but it also highlights how important , and little understood, this area of research is.

22. Tilman D: *Resource Competition and Community Structure*. Princeton Press; 1982.
23. Kazamia E, Aldridge DC, Smith AG: **Synthetic ecology – A way forward for sustainable algal biofuel production?** *J. Biotechnol.* 2012, **162**:163–169.
24. Elowitz M, Lim WA: **Build life to understand it.** *Nature* 2010, **468**:889–90.
25. Goers L, Freemont P, Polizzi KM: **Co-culture systems and technologies : taking synthetic biology to the next level.** 2014
26. Hong S, Pan Q, Lee LP: **Single-cell level co-culture platform for intercellular communication.** *Integr. Biol.* 2012, **4**:374.
27. Jeong H-H, Jin SH, Lee BJ, Kim T, Lee C-S: **Microfluidic static droplet array for analyzing microbial communication on a population gradient** *Lab Chip* 2015, **15**:889–899.
28. Mohan R, Sanpitakseree C, Desai A V., Sevgen SE, Schroeder CM, Kenis PJ a.: **A microfluidic approach to study the effect of bacterial interactions on antimicrobial susceptibility in polymicrobial cultures** *RSC Adv.* 2015, **5**:35211–35223.
29. Frimat J-P, Becker M, Chiang Y-Y, Marggraf U, Janasek D, Hengstler JG, Franzke J, West J: **A microfluidic array with cellular valving for single cell co-culture.** *Lab Chip* 2011, **11**:231–237.
30. Hong SH, Hegde M, Kim J, Wang X, Jayaraman A, Wood TK: **Synthetic quorum-sensing circuit to control consortial biofilm formation and dispersal in a microfluidic device** *Nat. Commun.* 2012, **3**:613.
31. Leung K, Zahn H, Leaver T, Konwar KM, Hanson NW, Page a. P, Lo C-C, Chain PS, Hallam SJ, Hansen CL: **A programmable droplet-based microfluidic device applied to multiparameter analysis of single microbes and microbial communities.** *Proc. Natl. Acad. Sci.* 2012, **109**:7665–7670.
32. Luo X, Tsao C-Y, Wu H-C, Quan DN, Payne GF, Rubloff GW, Bentley WE: **Distal modulation of bacterial cell–cell signalling in a synthetic ecosystem using partitioned microfluidics** *Lab Chip* 2015, **15**:1842–1851.
33. Ferrari BC, Binnerup SJ, Gillings M: **Microcolony Cultivation on a Soil Substrate Membrane System Selects for Previously Uncultured Soil Bacteria** *Microcolony Cultivation on a Soil Substrate Membrane System Selects for Previously Uncultured Soil Bacteria.* 2005, **71**:8714–8720.

34. Bollmann A, Lewis K, Epstein SS: **Incubation of environmental samples in a diffusion chamber increases the diversity of recovered isolates.** *Appl. Environ. Microbiol.* 2007, **73**:6386–6390.
35. Nichols D, Cahoon N, Trakhtenberg EM, Pham L, Mehta A, Belanger A, Kanigan T, Lewis K, Epstein SS: **Use of ichip for high-throughput in situ cultivation of “uncultivable” microbial species.** *Appl. Environ. Microbiol.* 2010, **76**:2445–2450.
36. Svenning MM, Wartiainen I, Hestnes AG, Binnerup SJ: **Isolation of methane oxidising bacteria from soil by use of a soil substrate membrane system.** *FEMS Microbiol. Ecol.* 2003, **44**:347–354.
37. Ling LL, Schneider T, Peoples AJ, Spoering AL, Engels I, Conlon BP, Mueller A, Hughes DE, Epstein S, Jones M, et al.: **A new antibiotic kills pathogens without detectable resistance.** *Nature* 2015, doi:10.1038/nature14098.
38. Aoi Y, Kinoshita T, Hata T, Ohta H, Obokata H, Tsuneda S: **Hollow-fiber membrane chamber as a device for in situ environmental cultivation.** *Appl. Environ. Microbiol.* 2009, **75**:3826–3833.
39. Kim HJ, Boedicker JQ, Choi JW, Ismagilov RF: **Defined spatial structure stabilizes a synthetic multispecies bacterial community.** *Proc. Natl. Acad. Sci. U. S. A.* 2008, **105**:18188–18193.
40. Moffitt JR, Lee JB, Cluzel P: **The single-cell chemostat: an agarose-based, microfluidic device for high-throughput, single-cell studies of bacteria and bacterial communities.** *Lab Chip* 2012, **12**:1487.
41. Choi WS, Ha D, Park S, Kim T: **Synthetic multicellular cell-to-cell communication in inkjet printed bacterial cell systems** *Biomaterials* 2011, **32**:2500–2507.
42. Drachuk I, Suntivich R, Calabrese R, Harbaugh S, Kelley-Loughnane N, Kaplan DL, Stone M, Tsukruk V V.: **Printed Dual Cell Arrays for Multiplexed Sensing** *ACS Biomater. Sci. Eng.* 2015, doi:10.1021/ab500085k.

** This study demonstrates inkjet printing of environmental biosensors. Two *E. coli* strains are modified to fluoresce in the presence of specific environmental chemicals. These bacteria are encapsulated by silk fibroin with the goal of protecting the *E. coli* when deployed into the environment.

43. Connell JL, Ritschdorff ET, Whiteley M, Shear JB: **3D printing of microscopic bacterial communities.** *Proc. Natl. Acad. Sci. U. S. A.* 2013, **110**:18380–5.

* The authors use multi-photon polymerization to 3D print microscale gelatin enclosures around bacterial communities.

44. Kim H, Du W, Ismagilov R: **Complex Function by Design Using Spatially Pre-Structured Synthetic Microbial Communities: Degradation of Pentachlorophenol in the Presence of HG(II).** *Integr. Biol.* 2011, **3**:126–133.
45. Wang Z, Wu X, Peng J, Hu Y, Fang B, Huang S: **Artificially constructed quorum-sensing circuits are used for subtle control of bacterial population density.** *PLoS One* 2014, doi:10.1371/journal.pone.0104578.
46. You L, Cox RS, Weiss R, Arnold FH: **Programmed population control by cell-cell communication and regulated killing.** *Nature* 2004, **428**:868–871.
47. Basu S, Mehreja R, Thiberge S, Chen M-T, Weiss R: **Spatiotemporal control of gene expression with pulse-generating networks.** *Proc. Natl. Acad. Sci. U. S. A.* 2004, doi:10.1073/pnas.0307571101.
48. Basu S, Gerchman Y, Collins CH, Arnold FH, Weiss R: **A synthetic multicellular system for programmed pattern formation.** *Nature* 2005, doi:10.1038/nature03461.
49. Wu F, Menn DJ, Wang X: **Quorum-Sensing Crosstalk-Driven Synthetic Circuits: From Unimodality to Trimodality.** *Chem. Biol.* 2014, **21**:1629–1638.
50. Marchand N, Collins CH: **Peptide-based communication system enables Escherichia coli to Bacillus megaterium interspecies signaling.** *Biotechnol. Bioeng.* 2013, doi:10.1002/bit.24975.
51. Chen AY, Deng Z, Billings AN, Seker UOS, Lu MY, Citorik RJ, Zakeri B, Lu TK: **Synthesis and patterning of tunable multiscale materials with engineered cells.** 2014, doi:10.1038/NMAT3912.
52. Zhang X, Reed JL, Fong SS: **Adaptive Evolution of Synthetic Cooperating Communities Improves Growth Performance.** *PLoS One* 2014, **9**.
53. Kerner A, Park J, Williams A, Lin XN: **A programmable escherichia coli consortium via tunable symbiosis.** *PLoS One* 2012, doi:10.1371/journal.pone.0034032.
54. Wintermute EH, Silver PA: **Emergent cooperation in microbial metabolism.** *Mol. Syst. Biol.* 2010, **6**:407.
55. Mee MT, Collins JJ, Church GM, Wang HH: **Syntrophic exchange in synthetic microbial communities.** doi:10.1073/pnas.1405641111.

* This work shows the construction of synthetic syntrophic communities with different complexities. It reveals principles of metabolic exchange that will be useful for constructing higher-order metabolic interactions in synthetic communities.

56. Shou W, Ram S, Vilar JMG: **Synthetic cooperation in engineered yeast populations.** *Proc. Natl. Acad. Sci. U. S. A.* 2007, doi:10.1073/pnas.0610575104.
57. Harcombe WR, Riehl WJ, Dukovski I, Granger BR, Betts A, Lang AH, Bonilla G, Kar A, Leiby N, Mehta P, et al.: **Metabolic resource allocation in individual microbes determines ecosystem interactions and spatial dynamics.** *Cell Rep.* 2014, doi:10.1016/j.celrep.2014.03.070.
58. Harcombe W: **Novel cooperation experimentally evolved between species.** *Evolution* 2010, **64**:2166–72.
59. Youk H, Lim WA: **Secreting and Sensing the Same Molecule Allows Cells to Achieve Versatile Social Behaviors.** doi:10.1126/science.1242782.
60. Weber W, Daoud-El Baba M, Fussenegger M: **Synthetic ecosystems based on airborne inter- and intrakingdom communication.** *Proc. Natl. Acad. Sci. U. S. A.* 2007, **104**:10435–40.
61. Goñi-Moreno A, Amos M, de la Cruz F: **Multicellular Computing Using Conjugation for Wiring.** *PLoS One* 2013, doi:10.1371/journal.pone.0065986.
62. Opitz M, Weber MF, Poxleitner G, Hebisch E, Frey E: **Chemical warfare and survival strategies in bacterial range expansions** doi:10.1098/rsif.2014.0172.
- * Genetically engineered three-strain consortia recapitulating standard ecological scenarios were analyzed systematically and with the aid of computational models.
63. Chen A, Sanchez A, Dai L, Gore J: **ARTICLE Dynamics of a producer-freeloader ecosystem on the brink of collapse.** 2014, doi:10.1038/ncomms4713.
64. Müller MJI, Neugeboren BI, Nelson DR, Murray AW: **Genetic drift opposes mutualism during spatial population expansion.** *Proc. Natl. Acad. Sci. U. S. A.* 2014, **111**:1037–42.
65. Gieg LM, Fowler SJ, Berdugo-Clavijo C: **Syntrophic biodegradation of hydrocarbon contaminants.** *Curr. Opin. Biotechnol.* 2014, **27**:21–9.
66. Bobadilla Fazzini RA, Bielecka A, Quintas AKP, Golyshin PN, Preto MJ, Timmis KN, dos Santos VAPM: **Bacterial consortium proteomics under 4-chlorosalicylate carbon-limiting conditions.** *Proteomics* 2009, **9**:2273–85.
67. Baghapour MA, Nasser S, Derakhshan Z: **Atrazine removal from aqueous solutions using submerged biological aerated filter.** *J. Environ. Heal. Sci. Eng.* 2013, **11**:6.
68. Horemans B, Breugelmans P, Hofkens J, Smolders E, Springael D: **Environmental dissolved organic matter governs biofilm formation and subsequent linuron**

- degradation activity of a linuron-degrading bacterial consortium.** *Appl. Environ. Microbiol.* 2013, **79**:4534–42.
69. Antoniou E, Fodelianakis S, Korkakaki E, Kalogerakis N: **Biosurfactant production from marine hydrocarbon-degrading consortia and pure bacterial strains using crude oil as carbon source.** *Front. Microbiol.* 2015, **6**:274.
 70. Bak F, Bonnichsen L, Jørgensen NOG, Nicolaisen MH, Nybroe O: **The biosurfactant viscosin transiently stimulates n-hexadecane mineralization by a bacterial consortium.** *Appl. Microbiol. Biotechnol.* 2015, **99**:1475–83.
 71. Ganesh Kumar A, Vijayakumar L, Joshi G, Magesh Peter D, Dharani G, Kirubakaran R: **Biodegradation of complex hydrocarbons in spent engine oil by novel bacterial consortium isolated from deep sea sediment** *Bioresour. Technol.* 2014, **170**:556–564.
 72. Mnif I, Mnif S, Sahnoun R, Maktouf S, Ayedi Y, Ellouze-Chaabouni S, Ghribi D: **Biodegradation of diesel oil by a novel microbial consortium: comparison between co-inoculation with biosurfactant-producing strain and exogenously added biosurfactants.** *Environ. Sci. Pollut. Res. Int.* 2015, doi:10.1007/s11356-015-4488-5.
 73. Do Nascimento M, Dublan M de los A, Ortiz-Marquez JCF, Curatti L: **High lipid productivity of an Ankistrodesmus-Rhizobium artificial consortium.** *Bioresour. Technol.* 2013, **146**:400–7.
 74. Maki M, Iskhakova S, Zhang T, Qin W: **Bacterial consortia constructed for the decomposition of Agave biomass.** *Bioengineered* **5**:165–72.
 75. Eiteman MA, Lee SA, Altman E: **A co-fermentation strategy to consume sugar mixtures effectively.** *J. Biol. Eng.* 2008, doi:10.1186/1754-1611-2-3.
 76. Zuroff TR, Xiques SB, Curtis WR: **Consortia-mediated bioprocessing of cellulose to ethanol with a symbiotic Clostridium phytofermentans/yeast co-culture.** *Biotechnol. Biofuels* 2013, **6**:59.
 77. Du R, Yan J, Li S, Zhang L, Zhang S, Li J, Zhao G, Qi P: **Cellulosic ethanol production by natural bacterial consortia is enhanced by Pseudoxanthomonas taiwanensis.** *Biotechnol. Biofuels* 2015, **8**:10.
 78. Kim S, Baek S-H, Lee K, Hahn J-S: **Cellulosic ethanol production using a yeast consortium displaying a minicellulosome and β -glucosidase.** *Microb. Cell Fact.* 2013, **12**:14.
 79. Minty JJ, Singer ME, Scholz SA, Bae C-H, Ahn J-H, Foster CE, Liao JC, Lin XN: **Design and characterization of synthetic fungal-bacterial consortia for direct production of isobutanol from cellulosic biomass.** doi:10.1073/pnas.1218447110.

****This work is an excellent example of how natural and engineered organisms can be put together and applied to a real-world problem.**

80. Kiely PD, Regan JM, Logan BE: **The electric picnic: synergistic requirements for exoelectrogenic microbial communities.** *Curr. Opin. Biotechnol.* 2011, **22**:378–85.
81. Logan BE: **Exoelectrogenic bacteria that power microbial fuel cells.** *Nat. Rev. Microbiol.* 2009, **7**:375–81.
82. Bourdakos N, Marsili E, Mahadevan R: **A defined co-culture of *Geobacter sulfurreducens* and *Escherichia coli* in a membrane-less microbial fuel cell.** *Biotechnol. Bioeng.* 2014, **111**:709–18.

**** Engineering of a synthetic microbial consortium for a microbial fuel cell to revealed interactions between fermentative organisms and exoelectrogens that influence the yield and feasibility of the MFC. This is another interesting application of consortia to bioenergy.**

83. Borrero J, Chen Y, Dunny GM, Kaznessis YN: **Modified Lactic Acid Bacteria Detect and Inhibit Multiresistant Enterococci.** [date unknown], doi:10.1021/sb500090b.
84. Hwang IY, Tan MH, Koh E, Ho CL, Poh CL, Chang MW: **Reprogramming microbes to be pathogen-Seeking killers.** *ACS Synth. Biol.* 2014, doi:10.1021/sb400077j.
85. Gupta S, Bram EE, Weiss R: **Genetically programmable pathogen sense and destroy.** *ACS Synth. Biol.* 2013, doi:10.1021/sb4000417.
86. Kotula JW, Jordan Kerns S, Shaket LA, Siraj L, Collins JJ, Way JC, Silver PA, Richard Kolodner by D: **Programmable bacteria detect and record an environmental signal in the mammalian gut.** doi:10.1073/pnas.1321321111.

*** We believe that engineering living therapeutics is an important future application of consortia and this paper shows function in a living, changing microbiome. They show integration of an engineered strain into the mouse gut and functional expression of a synthetic gene circuit that enables detection and memory of signals within the gut environment.**

87. Duan F, March JC: **Engineered bacterial communication prevents *Vibrio cholerae* virulence in an infant mouse model.** *Proc. Natl. Acad. Sci. U. S. A.* 2010, doi:10.1073/pnas.1001294107.
88. Chu T, Ni C, Zhang L, Wang Q, Xiao J, Zhang Y, Liu Q: **A quorum sensing-based in vivo expression system and its application in multivalent bacterial vaccine.** *Microb. Cell Fact.* 2015, doi:10.1186/s12934-015-0213-9.
89. Borman KL: ***Cladonia cristatella* (British Soldier).** <https://en.wikipedia.org/wiki/Cladonia#/media/File>, 2008.

90. Isaacs T: **Ear**. <https://www.flickr.com/photos/tbisaacs/3911558890/>, 2009.
91. Dziunka D: **Lake**. <https://www.flickr.com/photos/tbisaacs/3911558890/>, 2014.
92. Hollinger J: **Lungwort (Lobaria pulmonaria)**. <https://www.flickr.com/photos/tbisaacs/3911558890/>, 2007.
93. HighwaysEngland: **drainage ditch**. <https://www.flickr.com/photos/highwaysagency/61949>, 2006.
94. Park J, Kerner A, Burns M a., Lin XN: **Microdroplet-enabled highly parallel co-cultivation of microbial communities**. *PLoS One* 2011, **6**.

Figure 1

fungi alone

algae alone

complete lichen

during dessication, starvation, and irradiation

b.

macrolide antibiotics

beta-lactam antibiotics

Moraxella catarrhalis

Streptococcus pneumoniae

biofilm of *M. catarrhalis* & *S. pneumoniae*

c.

betaproteobacterium
motile

Chlorochromatium aggregatum

green sulfur
bacteria
photosynthesizer

light

motile
photosynthetic
community

d.

lichen fungi and algae

lichen bacterial symbionts

nutrients
vitamin B12
hormones
detoxification

e.

Smithella

hexadecane
activation

hexadecane

acetate
electrons

Methanosaeta concilii

methane

Methanocalculus

Figure 2

a. microfluidics

b. membranes

c. spatial patterning

Figure 3
a. metabolic cross-feeding

b. quorum sensing

c. conjugation

d. toxins

Figure 4 Mediation

b. bioenergy from complex feedstock

c. living therapeutics