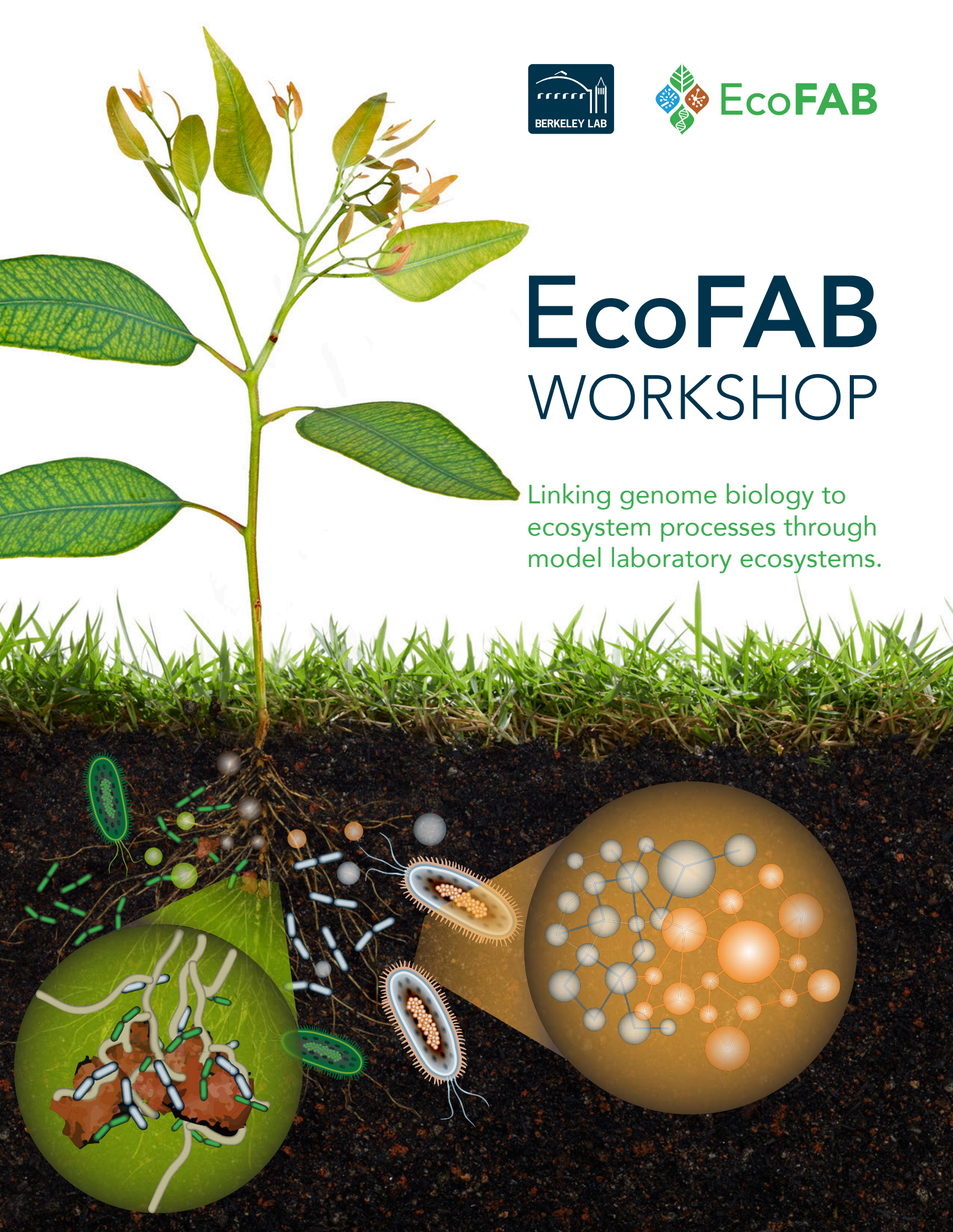

BERKELEY LAB

EcoFAB

EcoFAB WORKSHOP

Linking genome biology to ecosystem processes through model laboratory ecosystems.

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EcoFAB: Model Ecosystems Linking Genome Biology to Ecosystem Processes

EcoFAB

EcoFAB Workshop

March 22, 2016, Walnut Creek, CA

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Executive Summary

In recent years, our understanding of microbiomes has grown dramatically; we can observe the effects of microbiomes in a range of diverse environments, from soil on farms to inside the human gut. However, one of the major challenges for generalized understanding of microbiomes in these complex environments is standardizing the study systems and methods. Unlike the biomedical research community that has a wide range of model systems with different strengths and weaknesses, there is no agreed upon system for studying soil microbial communities and microbiomes, and thus, nearly every researcher in the field is studying a different set of microbes in a different soil system.

At the same time, recent advancements in metagenomics suggest that we will soon be able to assemble genomes for a large fraction of uncultivated microorganisms within microbiomes. The integration of this information along with biogeochemical and other measurements to accurately infer microbial community activities, stability and host interactions has the potential to transform our ability to produce sustainable energy and improve human and ecosystem health. Our understanding of microbial function in a community context lags behind our ability to identify and describe those microbes.

Bringing together communities of scientists to design and use ecosystem fabrication (EcoFAB) to construct reproducible and complex model ecosystems for the environment has the potential to greatly advance microbiome science. Importantly, these systems should be designed to be disseminated between scientists, bringing together diverse expertise and approaches to study the same controlled ecosystems to greatly accelerating our understanding of microbial communities.

This one-day workshop brought together a group of thought leaders with diverse expertise (microbial genomics, systems biology, soil science, microbial ecology, and bioinformatics) and included scientists from multiple national labs, industry, and academia. The goal was set to define initial model microbial communities for construction in reproducible, highly controlled, and instrumented model soil habitats and to determine the types of integrated measurements and standards required to accurately predict and model microbiome activities.

Workshop participants were organized into three break-out sessions with the goal of defining the following model ecosystems:

1. A model crop-microbe-soil to study growth promotion
2. A plant-microbe-soil system for studying carbon cycling
3. A soil-microbe-microbe system to study community assembly, activity, etc.

These breakout groups defined very similar model ecosystems and when convened to discuss the breakout outputs, the group coalesced around a single experimental system that could address research needs in crop plant growth promotion, carbon cycling, and community assembly. The selected system would use two related model grass species, *Brachypodium distachyon and Brachypodium sylvaticum*, that are relevant to both crops (for example, switchgrass and grains) and carbon cycling, grown in a simulated soil matrix made from synthetic clays established at the Joint Genome Institute inoculated with wild soil communities from UC Davis Russell Ranch or with defined but complex synthetic communities that promote growth of the plant. The same system could be used without the plant to focus on microbial interactions. The chemical parameters of selected soil in this system will be varied based on soil

measurements from Russell Ranch. Growth of *Brachypodium* at Russell Ranch will validate hypotheses about community formation and plant-microbe interactions in natural environments.

The EcoFAB workshop highlighted the need for robust model ecosystems that were reproducible and could be disseminated between labs as an important tool for examination and dissection of the ecology relevant to plant growth, soil carbon cycling, and understanding community dynamics.

Introduction

Increasingly we are recognizing the vital role microbiomes play in essentially all aspects of life on Earth. Environmental microbial communities largely govern global nutrient cycling, including the carbon and nitrogen cycles, and microbiomes associated with soils and plants are vital to agriculture. In recent years, investment from Federal agencies, including the Department of Energy, the US Department of Agriculture, and the National Science Foundation, and from agricultural companies has been targeted at understanding how microbial communities associated with crop plants can promote low-input, high-productivity agriculture. It has been known for some time that up to 50% of carbon fixed by plants is exuded by the roots. Likely a major portion of this is to attract and support beneficial microbes. Yet, significant research has been conducted on only a few key plant-microbe mutualisms, such as legume-rhizobia or mycorrhizal symbioses, which enable plants to access otherwise unavailable nitrogen and phosphorus sources. However, critical details are missing about even these common forms of plant-microbe symbiosis and about a wide range of other growth promoting microbial interactions within the rhizosphere. Microbiome work to understand these interactions holds great promise to reduce the amount of fertilizer and water required for agriculture, while displacing petroleum-based products and improving the sustainability and yield of current practices.

The need to better understand environmental microbiomes has been highlighted not only in scientific literature, but also by the U.S. Department of Energy and other federal research sponsors. DOE's Office of Science has highlighted its Grand Challenges, one of which is complex systems science across scales.[1] Many subsequent reports have highlighted understanding of microbial community dynamics in addressing key challenges in bioenergy development and climate. [2,3]

Beyond DOE, the U.S. Department of Agriculture has recognized the importance of microbiomes to enable low-input agriculture and to mitigate the effects of the changing environment. A recent Dear Colleague letter explains that novel research into the role of microbiomes in increasing food security and carbon sequestration is a necessary element to achieving the goals set forth in the USDA strategic plan.[4,5] The National Academies have noted the need for better understanding of microbial communities in recent reports, especially for communities found in the environment.[6] These efforts require detailed contextual information and model communities to be made accessible to the research community at large.

Tremendous advances in DNA sequencing now enable inexpensive sequencing bacterial genomes in high-throughput. As a result, we have accumulated and continue to accumulate vast amounts of sequence information from all types of organisms and environments. This, in principle, could provide the information necessary to make accurate predictions of microbial metabolism and fitness. However, we lack the necessary information on gene functions to make accurate predictions from environmental DNA sequence and we poorly understand the biology of soil organisms. Even for model organisms that have been studied for several decades, such as *E.coli*, 20% of genes still have no predicted function, and for another 10-20% we have only a very crude and general protein family information but lacking a precise enzymatic activity or function. The correlative research that has dominated the field may not be representative of the complex interactions happening in natural systems. The sequence-function gap is widely

[1] Biological and Environmental Research Grand Challenge Workshop Report DOE/SC-0135 (2010)
[2] BER Research for Sustainable Bioenergy DOE/SC-0167 (2013)
[3] BER Building Virtual Ecosystems Report DOE/SC-0171 (2014)
[4] http://www.nsf.gov/pubs/2016/nsf16058/nsf16058.jsp
[5] USDA Strategic Plan FY14-FY18 (2013)
[6] The Science and Applications of Microbial Genomics: Workshop, National Academies Press (2013)

recognized within the research community as one of the grand challenges in microbiology (and indeed, biology in general), and it is now well established and attempts to understand biological phenomena based on incomplete data that would certainly lead to erroneous conclusions. Moving beyond correlation to causality and design will require greatly improved understanding of gene functions and this will require moving from isolate cultures (where many genes have no apparent function) to more realistic environments and greatly improved understanding of the biology of soil organisms. These systems will enable hypothesis testing, for example to test the role of a microbe or biosynthetic pathway within a community, and also enable hypothesis generation for testing in field studies.

Despite the investment and promise of plant-associated microbiome research, we lack vital understanding of the plant-microbe-soil interactions that govern these communities. Most approaches aimed at improving our understanding of soil microbial communities are focused on examination of individual isolates or field studies of complex native communities. One promising research direction is constructing laboratory consortia, since these have the advantage that the constituent isolates can (in most cases) be characterized independently and even genetically manipulated to determine causal mechanisms. While these consortia systems allow researchers to test hypotheses about community interactions, the validity of extrapolating consortia-based findings to authentic 'field' communities has not been determined. Conversely, approaches for studying field microbial communities are challenging because they are so unconstrained and complex, and they often show irreproducible results such that definitive links between specific taxa and effects on plant growth or ecosystem function cannot be identified.

One solution to this challenge is to develop model soil ecosystems to allow for controlled, replicated laboratory experiments that can be validated in the field. Broad scientific community acceptance of a few of these model ecosystems would no doubt exponentially increase our understanding of microbial communities as a whole by focusing diverse expertise and capabilities on the same systems. By analogy, model organisms (e.g. mice) have been instrumental in determining the molecular and cellular biology of multicellular organisms. Indeed, human diseases are often studied using organisms that dramatically differ from humans (e.g. zebrafish) because they provide reproducible systems that can be manipulated in carefully controlled experiments in labs around the world.

New technologies are urgently needed to construct these model ecosystems with the capability of controlling the "microbial microenvironment"—the sum of all chemical and physical interactions impressed upon a cell by its biotic and abiotic environment. Studying these microenvironments by engineering and manipulating them and measuring their phenotypic outputs builds strong bottom-up understanding of the foundational chemical and genetic factors structuring microbial communities. While this may sound futuristic, advances in 2D and 3D fabrication of biomaterials could rapidly enable the construction of microbiomes with carefully controlled microenvironments and targeted cellular interactions. Critically, these synthetic microbiomes can be at a range of scales (e.g. aggregate scale, plant-scale) and will allow use of extant microbial and host genetics tools to test the roles of individual taxa and combinations of genes and microbes in their microenvironment and interaction contexts, thus establishing causal connections. Central to this approach will be unlocking the enormous treasure trove of genetic diversity currently beyond the reach of laboratory microbiologists by using microenvironment control to enable high-throughput culturing of the "unculturable".

The EcoFAB workshop defined these potential model soil ecosystems known as EcoFABs and determined the types of biotic and abiotic components integrated with the measurements and models required to accurately predict microbiome activities. An important consideration in the development of EcoFABs is that it be relevant to and used by the larger scientific community, ideally including laboratory, field and computational scientists. Thus, assemblies of communities

of scientists around specific EcoFABs, analogous to the existing Brachypodium and Arabidopsis communities is essential. The discussions and outputs of the workshop are discussed in the report-outs from each breakout session.

EcoFAB Component Selection

A great diversity of EcoFABs can be generated in principle and the details depend on the questions they are designed to address. Hence modular EcoFAB design is very desirable such that there would be 'core' EcoFABs relevant for a particular native ecosystem that could be disseminated between labs and then extended as part of the efforts of individual labs. Hence, our initial goal is to define the base system(s) that could be refined or expanded by the individual scientist to their specific needs. We envision that groups of scientists would work together and develop EcoFABs to address the needs of their community.

Field Site: The native ecosystem that is serving as the reference system is perhaps one of the most critical aspects of the EcoFAB design. We envision a stable source of model soil and microbes and a long-term experimental site for validation of our findings. Importantly, the "natural" reference community not only provides the source for the soil microorganisms (and potential metazoans) but also allows direct observation of the patterns of community organization and potential interactions (plant-microbe-soil). For example, which microbes regularly co-occur with each other, what is the proportion of saprotrophs, mutualists, and pathogens in a natural soil community? What are the temporal and spatial pattern alterations of the rhizosphere microbiome?

Figure 1. Aerial photo of Russell Ranch.

This workshop identified that the UC Davis Russell Ranch Sustainable Agriculture Facility (Figure 1) is well-suited as a field site for EcoFAB development. This 300-acre research farm has an ongoing 100-year experiment measuring long-term impacts of crop rotation, farming management practices, and water and nutrient inputs in organic and conventional production systems (Kong *et al.*, 2011). The Russell Ranch sustains continuous monitoring of environmental data through smart-sensor technology and remote sensing; maintains archived datasets of soil samples, farm operations, soil quality, crop yields, carbon sequestration, and greenhouse gases; and serves as training space for students and farmers. The Ranch houses 72 one-acre plots with rotations for different crop systems as well as native grasslands serving as control plots. Nested within each plot, there are 40 microplots, allowing researchers to

perform experimental work on a short-term basis or measure soil and biological properties that are relevant at multiple scales: from small (soil aggregate, rhizosphere) to large (landscape, ecosystem) scales. The UC Davis Russell Ranch is an excellent reference system for studying plant-soil-microbe interactions important for the understanding of carbon cycling, plant growth promotion, and soil microbiomes that are ecologically relevant and sustainable.

Figure 2. Photo of *Brachypodium distachyon.*

Model Plant: The EcoFAB concept is predicated on having as much control as possible over all inputs and organisms. Thus, it is desirable to use plants that are well characterized, genetically transformable, experimentally tractable and small enough to complete their lifecycle in the confines of a compact EcoFAB. Thus a wide range of plants could be used, including plants of the genera Arabidopsis, Setaria, and Brachypodium, among many others. For our initial experiments we have chosen to use two related model grasses, one that is annual (*Brachypodium distachyon*, Figure 2) and one that is perennial (*B. sylvaticum*). Having both annual and perennial model plants will facilitate investigation of soil C and microbiome dynamics under conditions that mimic farming with annual grains as well as perennial forage/biomass grasses. Both Brachypodium species form mycorrhizal symbioses (unlike Arabidopsis) and possess a typical grass root system. The genus Brachypodium occupies an intermediate evolutionary position in the grass family that allows it to be used as a general model for the grasses including grains and the grasses grown as biomass crops (Draper *et al.*, 2001; Steinwand *et al.*, 2013). The extensive genomic, genetic and experimental resources established for these model grasses (e.g. high-quality genome sequences, extensive sequenced mutant collections, large germplasm collections, and highly efficient transformation methods) will greatly accelerate studies to understand the plant's contribution to plant-microbiome interactions.

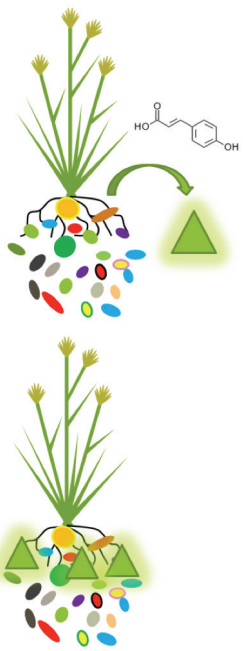

Figure 3. Illustration of using synthetic biology tools to construct reporter microbes (green triangle) that are chemiluminescent in the presence of a specific metabolite.

Model Bacteria and Genetic Tools: Bacteria should originate from the field site, either as isolates or intact communities, depending on the experimental goals. Complex communities can be obtained by filtering soil pore water and enriching the community under a diversity of growth conditions (Breidenbach *et al.*, 2015). As a parallel effort, a multi-condition isolation scheme (using both low and high-throughput approaches) should be conducted to get an isolate library. These isolates can be used to construct synthetic communities whose phenotypes can be compared to the native, enriched communities. Isolates and laboratory communities enable application of exometabolomics approaches to determine the metabolites uptaken and released by isolate organisms to inform possible crossfeeding and resource competition (Baran *et al.*, 2015) and then test predictions by monitoring community metabolism within the EcoFAB. In addition, a great advantage of EcoFAB versus native ecosystems is the ability to use genetic tools and mutants to enable the discovery of important genetic determinants of ecological processes. For example, the genetic tools being developed at Berkeley Lab can be applied to these isolates to gain a mechanistic understanding of their roles in microbial and plant interactions (Deutschbauer *et al.*, 2011; Firrincieli *et al.*, 2015; Lundberg *et al.*, 2012). These approaches include

random barcode transposon site mutagenesis coupled to sequencing to examine the fitness of all nonessential genes under *in situ* conditions and a complementary approach, Dub-Seq that enables examination of the impact of gene overexpression on organismal fitness and therefore can be used to characterize essential genes. Early stage technologies will provide additional tools for manipulation and visualization of the community including as phage engineering to selectively manipulate individual members of a complex microbiome, and reporter constructs for *in situ* spatial visualization of microbes in their native environment (for example, in association with plant roots, Figure 3). Specifically, work is underway at the Joint Genome Institute to develop chemiluminescent plant growth promoting bacteria to enable their visualization within the root system. Taken together, these approaches will enable the transition from correlative studies of microbiome structure to predictive models based on a detailed molecular understanding of microbe-plant interactions (Figure 4).

Model Fungi: Arbuscular mycorrhizal fungi (AMF) represent one the most ancient growth promoting plant-microbe symbioses and are present in >72% of angiosperm species (Parniske, 2008). As such, they represent the natural condition under which most other plant-microbe interactions occur. For this reason, including AMF is an important component of the EcoFAB design. Despite this, AMF present many challenges for a model system. They are obligate biotrophs and cannot be cultured in the absence of a host, key details of their sexual cycle and nuclear condition remain unknown, and their large genomes (up to 150 Mb) have proved highly challenging to sequence (Boon *et al.*, 2015; Tisserant *et al.*, 2013). Thus, tractability is a key concern for selecting an AMF for inclusion in EcoFAB. For this reason, strong consideration should be given for selection of a model AMF species, such as *Rhizophagus irregularis*, for which a complete genome exists and spores can be easily obtained from existing AMF culture collections (such as INVAM - The International Vesicular Arbuscular Mycorrhizal Collection). However, due to the promiscuous nature of AMF associations and the nearly global distribution of some AMF taxon, it is likely that a model AMF taxon such as *R. irregularis* is already present at Russell Ranch or, if not, that the interaction of *R. irregularis* with *Brachypodium sp*. and soil bacteria will still be a useful representation of the natural AMF community.

Figure 4. Illustration of plant microbe metabolite exchange.

Other Soil Organisms: Small fauna (e.g. arthropods, annelids, mollusks and nematodes), microbial eukaryotes and protists, RNA and DNA viruses associated with the rhizosphere of field Brachypodium are to be screened and isolated for their introduction to the artificial environments in different combinations to test for their influence in the development of the model plants as well as their influence in the overall microbial makeup of the ecosystem, i.e. changes in bacterial, fungal and phage populations. These fauna represent an important addition to EcoFABs in that they play critical roles in soil carbon cycling and other nutrient cycling in the rhizosphere. For example, maceration of plant debris by soil fauna greatly accelerates microbial decomposition, and the predation and subsequent digestion of soil microbes by nematodes can mobilize nitrogen and other nutrients enhancing plant growth (Neher, 1999).

Model Soil: Soil is one of the most difficult control aspects of an EcoFAB design. Field soils are generally unsuitable for controlled experiments for several reasons: they are undefined, they are not reproducible, they are difficult to sterilize without negatively changing soil chemistry, and they perform poorly in pots/containers largely due to poor drainage. While acid washed quartz sand is often used because it is inert, stable and reproducible, it is still undesirable because it has weak cation exchange capacity and lacks pores, both of which negatively impact plant health (Hendershot & Duquette, 1986; Jiang *et al.*, 2009). A kiln-fired ceramic substrate manufactured as a soil amendment for golf courses offers an attractive alternative. This material is stable, reproducible, easily sterilized, and has a high percentage of pore space and high cation exchange capacity (Steinberg *et al.*, 2005). In addition, this substrate contains no organic carbon which will facilitates carbon flux analyses. While this substrate does not possess all the properties of a field soil (e.g. aggregation of small particles and soil organic matter) it is a robust, reproducible reagent that will allow an EcoFAB to be standardized and reproduced in any laboratory. This said, an important research direction for EcoFABs is the development of synthetic soils. These can be a blend of acid washed quartz sand, defined organic amendments and synthetic clays to match the composition at Russell Ranch. While the preparation of such synthetic soils that accurately recapitulate native soils is an important long-term goal, in the short term combinations of simpler materials like the kiln fired ceramic substrate and gamma irradiated native soils provide viable starting points for the construction of EcoFABs.

Growth Chambers: There are many relevant examples of rhizotrons that provide starting points for the design of EcoFABs. 3-D printing technologies have the potential to greatly enable the construction of EcoFABs either through direct fabrication or through printing molds used to cast PDMS or other materials. For example, the root chip system which uses a PDMS chamber affixed to a glass slide space (Figure 5A) (Grossmann *et al.*, 2011). Its plant reservoir is sealed with agarose gel, and the roots can penetrate the gel and grow along the observation chamber be imaged using microscopy. Inlets and outlets enable the contents of the chamber to be changed for example in the case of hydroponic growth.

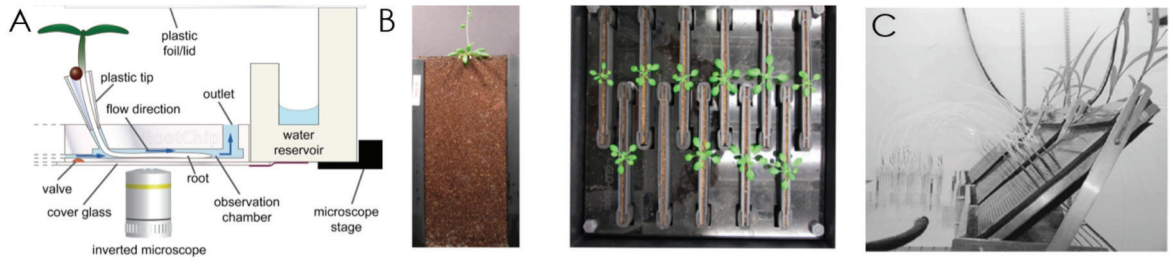

Figure 5. Rhizotrons designs that will be useful for EcoFAB construction. (A) Root-Chip (Grossmann *et al.*, 2011). (B) GLO-Roots platform (Rellán-Álvarez *et al.*, 2015). (C) Minirhizotron (Wang *et al.*, 2004).

Another example is the GLO-Roots system recently reported that uses a 2 mm thin sheet of soil between two sheets of polycarbonate plastic enabling the cultivation of plants such that their root system can be visualized especially for engineered plants expressing luciferase (Figure 5B) (Rellán-Álvarez *et al.*, 2015). Plastic racks hold the rhizotrons vertically and further protect the roots from light. These rhizotrons and rack are placed in a black tub and a small amount of water is added to the bottom to maintain moisture in the rhizotrons during plant growth. The volume of soil in the rhizotrons is similar to small pots commonly used for Arabidopsis growth

and supports growth of Arabidopsis throughout its entire lifecycle. One final rhizotron system that provides additional capabilities for EcoFABs construction is the Minirhizotron (Figure 5C) (Wang *et al.*, 2004). In this case, a portion of the rhizotrons plate contains small tubes positioned within the rhizotron connected to a manifold enabling spatially defined introduction and sampling of the rhizotrons contents. Integration of these three designs could provide a powerful EcoFAB design enabling both control of bulk flow through the chamber, spatially defined chemiluminescence imaging of the root system (and microbes, Figure 6), and the ability to sample and add microbes and materials to the root system in a spatially defined manner. All of this could be done while simultaneously imaging the rhizotrons contents using spectroscopic techniques. We also envisioned a range of advanced sensors could be integrated into the EcoFABs. These could be spatially defined micro-electrodes, opcodes and even wireless microdevices which together can provide near real time information on the environmental conditions within the chamber. This can be extremely powerful when integrated with the rhizotrons technologies described above. For example, arrays of pH sensors within an EcoFAB can be used to indicate microbial growth relative to the growing chemiluminescent root system while Minirhizotron sampling and sequencing through mass spectrometry analysis can identify the organisms and metabolites within this same region. It is important to note that while these examples are focused on plant-soil-microbe systems, the plant can be omitted to focus on soil-microbe interactions. A great advantage of using 3-D printing is that it can provide a great deal of flexibility enabling EcoFABs to be scaled appropriately for the question being asked. We can envision designs for EcoFABs be disseminated such that scientists around the world would be able to easily leverage each other's designs and local 3-D printing facilities to rapidly construct EcoFABs relevant to their question and may also be useful for educational purposes. For example: (http://rr-lab.github.io/GLO-Roots/).

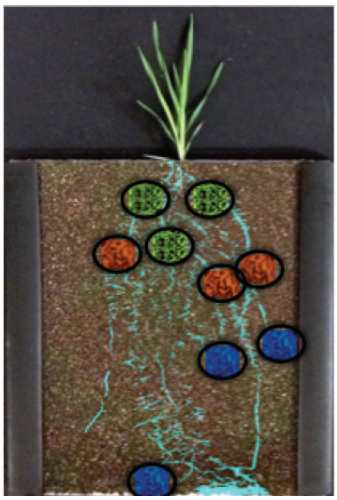

Figure 6. Illustration of EcoFAB containing chemiluminescent plant and reporter microbes signaling the presence of different metabolites from root exudates.

EcoFAB Characterization and Validation: Another important component of reproducible experiments is reproducible and standardized measurement technologies. There is a vast array of technologies which could be deployed within the EcoFAB context. Several methods were proposed for characterization of microbial community membership, activity and metabolism and are summarized briefly below:

Measurements of community structure, gene expression, protein expression and metabolic potential:

- Standard Illumina-based tag sequencing of marker genes including 16S rRNA and fungal Internal Transcribed Spacer (ITS) regions.
- Shotgun sequencing with Illumina, Pacific Biosciences or Oxford Nanopore.
- NanoStrings, RNA-seq to monitor gene expression.
- Standard proteomic methods can be used to characterize protein expression from biomass collected from the EcoFAB.

Assessment of active populations, metabolic flux, metabolic exchange and nutrient cycling during EcoFAB operation:

- 16S rRNA vs 16S rDNA sequencing to identify dormant taxa.
- Stable isotope probing (SIP), in which a substrate containing an isotopic label such as ^{13}C or ^{15}N is added to the community such that members able to take up and metabolize the substrate will incorporate the label into their nucleic acids. Isolation and separation of the heavy and light nucleic acids followed by sequence-based characterization enables identification of those microbes.
- BioOrthogonal NonCanonical Amino acid Tagging (BONCAT) coupled with Fluorescence Activated Cell Sorting (FACS), in which synthetic amino acids are taken up by metabolically active cells which can be rendered fluorescent by click chemistry and physically isolated for sequence-based characterization.
- D_2O coupled with Raman spectroscopy and microfluidic separation to isolate metabolically active organisms incorporating label into their lipids, followed by sequence-based metabolomics characterization.
- Electrochemical sensors can be included in the EcoFAB to provide near real-time analysis of the wide array of chemical parameters including pH, oxygen and electronic transfer. One exciting possibility is to use immobilized microbes that transfer electrons to the surface in response to specific environmental conditions.
- Optodes provide another approach for measuring chemical conditions within the EcoFAB and are well suited for measuring parameters such as oxygen.
- Exometabolomics provides a powerful tool for characterization of the metabolic transformations happening within the EcoFAB. This can be performed on spatially sampled regions of the chamber as well as the inflow and outflow to determine which metabolites are being produced and consumed within the various regions. Methods for analysis can include standard methods such as liquid chromatography tandem mass spectrometry and gas chromatography mass spectrometry but could also include high throughput laser desorption ionization methods that are particularly suited for very small sample volumes.
- Spectroscopic methods such as Raman and FTIR which enable analysis of biopolymer composition.
- Enzyme activity assay either using surrogate substrates coupled with colorimetric or fluorescence analysis or native substrates with mass spectrometry analysis can provide important insights into the enzymatic activities occurring within the EcoFAB.

Synthetic biology and genetic tools:

- High throughput genetics can be used to construct plant and microbial mutants for discovery with genes responsible for important phenotypes such as plant genes selecting for beneficial microbes. Mutant fitness analysis using Tn-SEQ and dub-SEQ can be used to discover microbial genes conferring fitness under specific environmental conditions or space responsible for ecological interactions.
- Synthetic biology tools can be used to introduce biosynthetic pathways to test their ecological function, for example, the production of secondary metabolites associated with intraspecific interactions, plant growth promotion, nutrient mobilization. It is also possible to construct reporter systems that will emit chemiluminescence in response to abiotic or biotic conditions of interest.

Methods of characterizing EcoFAB endpoints:

- X-ray techniques enable diverse analyses of the resulting EcoFAB contents. For example, x-ray microtomography can be used to create a three-dimensional reconstruction of the pore structure within the EcoFAB. X-ray absorptionspectroscopy can localize minerals and even measure at the nanoscale the absorption of metabolites and biopolymers onto mineral surfaces.
- Mass spectrometry imaging can be used to localize biomolecules especially metabolites and elements at the conclusion of an EcoFAB study. For example nanoSIMS can locate specific elements with 50 nanometer resolution providing vital information on the localization of stable isotopes within individual bacteria. Soft desorption ionization mass spectrometry approaches can be used to localize metabolites within the soil. DESI, NanoDESI, LAESI, and MALDI (Fang & Dorrestein, 2014) are all examples of technologies that can be used to localize metabolites within EcoFAB contents. A related technology, nanostructure-initiator mass spectrometry (Deng *et al.*, 2015; Northen *et al.*, 2007) has been used to image both enzyme activity and metabolites from fungal growth on switchgrass and is shown in Figure 7. NIMS imaging has sufficient spatial resolution to image fungal hyphae and is effective at localizing isotope incorporation given that high resolution mass spectrometers are used in this application.

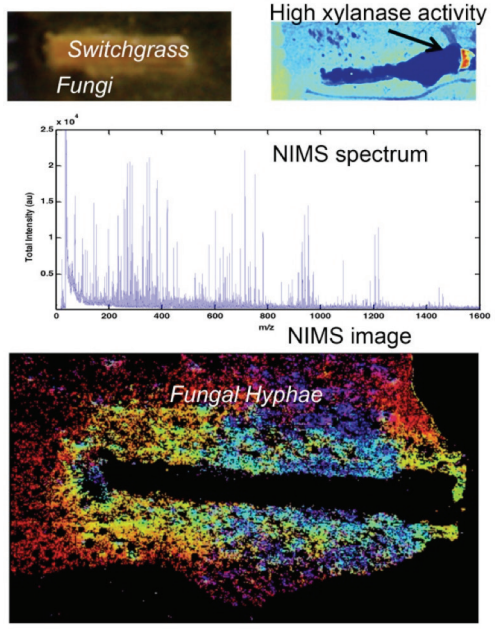

Figure 7. NIMS imaging of enzyme activity and metabolites from *Aspergillus* growing on switchgrass showing the rich mass spectra obtained.

EcoFAB Modeling and Data Analysis: The only way to derive robust and generalizable design principles for soil communities is to underpin this effort by predictive theoretical models. We will first decide on the community properties that will be most beneficial to predict (e.g. abundance distribution, diversity as a function of distance, robustness to perturbations, resilience). Once these are decided on, and depending on the types of data which are available in a repeatable and abundant fashion, the right modeling framework will be chosen, e.g. flux-based vs trait-based, coarse-grained vs spatially explicit, statistical vs mechanistical.

Multiple aspects of EcoFABs will require different modeling approaches. Gene and functional distributions can be understood in terms of population genetics models, while organism abundances and interaction distributions require ecological and game theory models. Integration of abundance and spatial information will require integrating plant development models with the population genetics and ecological models mentioned above. Integration of metabolic and chemical data with genomic content information can be achieved with multi-species flux based models. Ultimately, EcoFAB will produce a multi-scale modeling approach that combines fine- and coarse-grained methods on data from a common experimental platform. Moreover, key plant and microbial traits and environmental constraints can become incorporated into large-scale modeling efforts (e.g. CENTURY model).

EcoFAB Use Cases

Harnessing beneficial microbes to enable low-input high productivity crop.

We face a confluence of environmental and social challenges that will make it difficult to provide for a rapidly growing population while safeguarding vital environmental ecosystem processes. Of particular concern is the enormous gap between projected agricultural production and projected demand. For example, it has recently been estimated that agricultural productivity must be increased by 60% by 2050 to support humanity (Hatfield & Walthall, 2015). Further complicating matters are the uncertain challenges posed by climactic variability coupled with soil erosion and the unsustainability of current resource intensive agricultural practices.

Currently, nearly all suitable land is already under cultivation and it is highly undesirable to clear additional land. In fact, farmland is actually being lost due to urbanization and degradation (e.g. erosion, salinization and nutrient depletion). For the better part of the last century, the widespread application of chemical fertilizers has led to crop yields increasing fast enough to meet growing demand. Unfortunately, for the major grain crops, yield increases have slowed dramatically or leveled off in the past 10 years, and we are approaching the maximum genetic yield potential in the major staple crops. New mechanisms to increase yield that are complementary to current plant breeding and agronomic practices are needed to satisfy the demand for agricultural products such as biofuels. *Just as we have learned that the human microbiome—the collection of resident microorganisms—plays an enormous role in health and disease (Althani et al., 2016), we are beginning to appreciate the crucial role that microbiomes play in healthy and productive soils.*

EcoFAB will play a fundamental role in advancing our understanding of plant microbiomes by providing the tools required to investigate the mechanisms how plants enhance their abiotic stress tolerance and attract and maintain beneficial microbes to increase yield under sub-optimal conditions. Specifically, by enabling control of the soil environment and both the plant and microbiome genetics coupled with systems biology and advanced imaging technologies, it will be possible to gain new foundational insights into the mechanisms how plants genetically select beneficial microbes and how these beneficial microbes improve plant productivity and environmental tolerance. These insights will provide a more complete understanding of plant genomics, and also will critically enable the development of new plant cultivars coupled with specific microbiomes to grow on sub-optimal land. For example, selectively breeding plants can maintain beneficial microbiomes to improve nutrient utilization and water use efficiency.

Harnessing plants and microbes to predict carbon cycling and enabling approaches to build soil carbon.

Central to increasing agricultural and ecosystem productivity is understanding soil carbon cycling. This is because soil carbon is critical to agricultural productivity since it supports diverse beneficial microbiomes and retains water and vital nutrients. Years of poor land management including extractive agricultural practices have released massive amounts of soil carbon and degraded vast swaths of farmland, some to the point that it is no longer suitable for agriculture (Turner *et al.*, 2016). In fact, it is now thought that 30-70% of soil carbon has been lost and 50% of agricultural land is moderately or severely degraded. A deep mechanistic understanding of soil carbon cycling is desperately needed to enable development of approaches that build soil carbon and beneficial microbiomes to increase the sustainable productivity of degraded and even marginal soils.

Plants are also the major carbon inputs into soils where they are remineralized through the activities of microbes and soil fauna. The soil fauna are highly diverse multicellular organisms that play critical roles in soil fertility and global carbon cycling. For example, megafauna (e.g. earthworms) drive bulk mixing of soils and mesofauna can accelerate decomposition of plant litter (Hättenschwiler & Gasser, 2005). Thus foundational knowledge of metazoan genomes, their basic metabolic processes, and relationships with plants and environmental microbes are important in accurately predicting ecosystem responses and carbon cycling.

While there is huge industrial interest in identification of beneficial microbes, these efforts are focused on large-scale field screening rather than determination of the mechanisms for driving the benefit. Similarly, most academic studies have been performed either on isolated microbes or fully complex environmental communities, both of which have major challenges. Since, studying individual microbes in liquid culture is a vast extrapolation from their natural habitat, it is not surprising that we do not observe any function for approximately half of the genes in a bacterial genome under these conditions. The same is likely true for a large number of plant genes, which are presumably dedicated to selecting for and maintaining beneficial microbiomes. Yet, because of the extreme complexity and undefined nature of the soil microbiome, it is very difficult to determine the functions of specific genes and plant-soil-microbe interactions under field conditions. Thus we know very little about the molecular interplay of microbiomes and their host interactions. However, some labs, including workshop participants Mary Firestone, Jose Dinneny, John Vogel, Karsten Zengler, Sur Paredes and Jeff Dangl all have shown successes in model communities making us optimistic about the promise of laboratory soil ecosystems.

To address these challenges, it is important to develop EcoFABs that, in conjunction with field studies are designed to accurately recapitulate relevant soil processes. These can range in complexity from simple synthetic soil with defined microbes to native soils including microbes as well as metazoans and plants. Integration of these approaches with soil metabolomics and spectroscopy will provide unprecedented insights into soil organic matter formation and cycling. Through controlled manipulations of the plants metazoans and microbes it will be possible to determine the biotic controls on the soil organic matter formation. For example, it will be possible to leave out a particular soil organism and characterize the fact on carbon dynamics within the EcoFAB. Another example would be changing the mineral composition of the soil and using x-ray spectroscopy and nanoscale mass spectrometry imaging technologies to investigate which microbial metabolites adsorb onto the surfaces. Further it will be possible to use synthetic biology tools to test the role of specific genes and pathways in mediating important soil carbon cycling processes and make reporter microbes that would become florescent or luminescent under a particular environmental condition of interest enabling them to be used as diagnostic probes for *in situ* processes.

Since there are a great range of relevant length scales necessary to investigate carbon cycling, we envision EcoFABs spanning relevant length scales. This would include EcoFABs designed to study microbial processes on mineral services at the micro aggregate scale in conjunction with high resolution imaging technologies. At the upper end of the scale, it would be the construction and use of Ecotrons with meter scale soil monoliths and plant canopies that are instrumented such that both aboveground and belowground processes can be manipulated and monitored (Roy *et al.*, 2016). However, the bulk of work will likely be performed at the centimeter scale with benchtop EcoFABs designed to investigate the interactions of microbes, soils and metazoans with individual plants.

The EcoFABs will also serve as testbeds for the environment, enabling prototyping approaches and interventions to be used together to develop predictive models and effective approaches to

harness plants and beneficial microbiomes to build soil carbon and increase agricultural productivity. Through the creation of EcoFABs spanning multiple spatial scales it will be possible to test scaling predictions to better understand which processes can accurately be scaled and predicted from smaller scale measurements.

Summary and Conclusions

Feeding and fueling humanity while managing vital ecosystem processes in the face of changing environments poses a grand challenge to humanity. Addressing these challenges will require tremendous advances in our understanding of soil ecosystems to enable accurate predictions and inform the development of sustainable solutions. Major challenges for generalized understanding of microbiomes in these complex environments is developing standardized ecosystems. Critical to developing EcoFABs is bringing together communities of scientists around specific native ecosystems and questions to establish sharable microbial communities. Community acceptance of a few laboratory ecosystems analogous to gnotobiotic mice in the biomedical field would greatly advance our understanding of environmental microbiomes by bringing to bear the diverse tools and expertise of the larger scientific community.

This workshop brought together thought leaders from academia, national labs, and industry to discuss the experimental considerations required to build the first model laboratory ecosystems. These discussions were focused on model ecosystems for studying environmental microbiomes, specifically, soil microbe-microbe interactions, soil-plant-microbe interactions for investigation of soil carbon cycling and low input sustainable crop production. It was determined that selecting a native ecosystem was the essential first step for building EcoFABs for laboratory investigation of these processes by providing the biotic and abiotic components and enabling validation of the EcoFABs through comparison of laboratory and field studies.

The UC Davis Russell Ranch facility was identified as an excellent site to support the construction of the first generation of EcoFABs. Specifically, bacteria, phage, fungi, soil metazoans and other soil organisms can be isolated from this site. Where possible synthetic biology and genetic tools can be developed for these organisms to test causal mechanisms and investigate in situ processes using luminescence and fluorescence reporter systems. Equally important is the characterization of the soil environment and composition. The soils at Russell Ranch have already been extensively characterized and this information can be used to prepare synthetic soils or simply used either following sterilization or directly. In addition this site is already actively monitored for a diverse array of environmental parameters. While the core EcoFAB labs will focus on developing a sharable community using Davis soil, likely other labs will also want to do the same for their particular soil or model field. Providing methods that would allow others to create EcoFAB compatible communities would be a major advance and allow some means of standardizing the development of sharable communities.

Brachypodium distachyon was identified as an excellent model plant system in that it is relevant for both biofuel crops and major grain crops and yet is compact and has a number of genetic resources available making it tractable to meet EcoFAB objectives. Given these raw components the last portion of the discussion was focused on how to assemble them in devices that enable imaging spatial manipulation and spatially defined sampling. Several examples of rhizotrons provide great starting points for the development of EcoFABs especially the Glo-Root system and mini-rhizotrons. Integrating these systems with microfluidics was identified as a powerful opportunity to develop a system where microbes could be selectively introduced at

specific locations around the roots and spatially defined samples could be obtained for analysis of metabolites and microbes while simultaneously using chemiluminescence to monitor the root microbe soil environment.

Overall, participants in this workshop agreed that there is both significant need and interest in developing EcoFABs. Critical to the success of EcoFAB approach depends on the standardization, reproducibility and dissemination of model ecosystems. Initial experiments should focus on demonstrating that these laboratory ecosystems can be reproducibly assembled and achieve the same community composition, chemical composition, and overall activities. Once demonstrated it will be important to make the raw resources available to other laboratories and validate that they can recapitulate the same community with the same behavior. Berkeley lab was identified for the development and initial testing and then once reproducibility is established Karsten Zengler at UCSD, Jose Dinneny at the Carnegie Institution for Science and ideally others will serve as secondary locations for validation of dissemination. Since it is undesirable for academic labs to maintain culture collections and other materials relevant for the construction of model ecosystems, it will be important to involve industry. Strong industrial interest would greatly enhance the possibility that a culture collection would be incentivized to maintain collections of organisms. A broad range of scientists with diverse expertise are interested in model ecosystems, it is important to first develop the core EcoFABs and that the collections of organisms would be maintained by culture collections. This would provide researchers around the world the same starting point for their individual studies with every expectation that they will develop unique applications and resources. For example, one laboratory may be particularly interested in fungi and create a larger collection of fungi. Finally, there was great enthusiasm for the concept of having a conference dedicated to these model ecosystems to provide an important venue for scientists around the world to share learnings on their model ecosystems and to link them to native ecosystems.

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APPENDICES

Appendix 1. Workshop Agenda

March 22, 2016

9:00am	Opening and Introductions
9:15am	Karsten Zengler: *Multi-dimensional Interactions Define Dynamics in Microbial Communities*
9:45am	Barbara Campbell: *Microbial Community Structure and Function in Atlantic Coastal Waters*
10:15am	Break
10:30am	Chuck Pepe-Ranney: *Tracking Carbon Into and Through the Soil Microbial Community with DNA-SIP*
11:00am	Sur Herrera-Paredes: *Who, What and Eventually How: Bacterial Combinatorics for Plant Phenotypes*
11:30am	Breakout Session Assignments
11:45am	Working Lunch
1:45pm	Break
2:00pm	Define 3 Model Systems
3:30pm	Summary, Next Steps & Leads
4:00pm	Close

Appendix 2. Workshop Charge Questions

The following are some key aspects of the EcoFABs to be addressed. Please determine the minimum that is required for the 1.0 design and what should be the target for the 2.0 design.

Ecology

Is it important to base the model ecosystems on natural ecosystems?

What are the attributes of a native system to be selected for modelling in the laboratory?

What native systems will be used as a reference for the model ecosystems?

How will validity of the model ecosystem be determined?

Soil Science

What soil or model soil should be used?

How is the soil prepared?

Does it need to be sterilized? If so, how?

How can it be reproduced?

How will the soil be disseminated?

Microbiology

What are the criteria for selecting microbes to include in the model ecosystems?

Recommendations for key taxa to be included?

How do we know we have the right microbes?

How many different microbes are required?

How will these microbes be maintained and disseminated?

Plants

What is the criteria for selecting the model plant?

What would be the initial plant to use?

How is the plant maintained and disseminated?

Fabrication

What is the appropriate scale for cultivation?

Which aspects of the EcoFAB should be controlled?

What should be physically or chemically manipulated?

How can we design the EcoFAB to be both reproducible and flexible?

How is the design standardized and disseminated?

Sampling, Sensing and Imaging

Rank the most important parameters that should be monitored?

What needs to be sampled and at what resolution?

What needs to be imaged? What kind of imaging and what resolution?

Does this need to be standardized?

Sample Measurement

What would be the most informative sample measurements?

Does this constrain the other aspects of the design?

Implementation

What information is required for a lab to effectively set-up an EcoFAB?

How do they know that they have done it correctly?

What forum is needed to share designs?

Data Capture and Sharing

What is the minimum set of parameters that must be recorded to reproduce an EcoFAB state?

How is the information reported?

Modeling

Any special aspects of the EcoFAB that should be constrained to improve computational modeling efforts?

Appendix 3. Workshop Participants

Name	Institution	Role
Adam Deutschbauer	LBNL	Participant
Adam Rivers	LBNL	Participant
Alvin Tamsir	Pivot Bio	Participant
Barbara Campbell	Clemson University	Speaker
Ben Bowen	LBNL	Participant
Ben Brown	LBNL	Participant
Charles Pepe-Ranney	Cornell University	Speaker
Daniel van der Lelie	FMC Corporation	Participant
Eoin Brodie	LBNL	Participant
Hector Garcia Martin	LBNL	Participant
Javier Ceja Navarro	LBNL	Participant
Jennifer Pett-Ridge	LBNL	Participant
Jenny Mortimer	LBNL	Participant
Jian Gao	LBNL	Participant
John Vogel	LBNL	Participant
Jorge Rodrigues	UC Davis	Participant
Kabir Peay	Stanford	Breakout Lead: Growth Promotion
Karsten Zengler	UC San Diego	Speaker
Katherine McMahon	University of Wisconsin	Participant
Katy Christiansen	LBNL	Participant, Author

Kirsten Hofmockel	PNNL	Participant
Margaret McFall-Ngai	University of Hawaii	Participant
Mary Firestone	UC Berkeley	Breakout Lead: Carbon Cycling
Matt Smith	UC Berkeley	Participant
Matt Traxler	UC Berkeley	Participant
Matthew Francis	UC Berkeley	Participant
Michalis Hadjithomas	LBNL	Participant
Michi Taga	UC Berkeley	Participant
Natalia Ivanova	LBNL	Participant
Nikos Kyrpides	LBNL	Co-Chair
Peter Nico	LBNL	Participant
Romy Chakraborty	LBNL	Participant
Steve Singer	LBNL	Participant
Sue Celniker	LBNL	Participant
Sur Herrera-Paredes	University of North Carolina	Speaker
Susannah Tringe	LBNL	Co-Chair
Tanja Woyke	LBNL	Breakout Lead: Community Assembly
Trent Northen	LBNL	Co-Chair
Yasuo Yoshikuni	LBNL	Participant

Appendix 4. Other Contributors

Jose Dinneny	Carnegie Institute	Contributor
Jeff Dangl	University of North Carolina, Chapel Hill	Contributor