Title
FABRICATED ECOSYSTEM WORKSHOP BRIDGING LABORATORY TO FIELD SCIENCE WORKSHOP REPORT

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FABRICATED ECOSYSTEM WORKSHOP
BRIDGING LABORATORY TO FIELD SCIENCE

WORKSHOP REPORT

Lawrence Berkeley National Laboratory (Berkeley Lab) | February 25, 2020

EcoPOD

EcoFAB

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Front Cover: Esther Singer works on collecting soil samples from the newly installed EcoPOD at Berkeley Lab. Image credit: **Marilyn Sargent** (Berkeley Lab).

The EcoPOD workshop was supported by EGSB divisional funds.

EcoPOD

EcoFAB

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EXECUTIVE SUMMARY

Lawrence Berkeley National Laboratory (Berkeley Lab) scientists held a workshop at the DOE BER Genomic Sciences PI meeting on the use of Fabricated Ecosystems in Washington D.C. on February 25th, 2020. Ecosystem fabrication is an approach to creating controlled microbial, soil and plant ecologies within a laboratory setting that enable discovery and dissection of environmental variables, activities, and interactions. At Berkeley Lab, we are developing two systems which span spatial and temporal scales — the EcoFAB and the EcoPOD. The participants of the workshop discussed the potential applications and challenges of using fabricated ecosystems as tools to tackle BER-relevant scientific questions. Specifically, they identified (**1**) research challenges that would benefit from ready access to this infrastructure (**2**) identified and prioritized technical challenges that currently limit these systems and (**3**) discussed how to use them to bridge the gap between lab and field research.

To ensure that the fabricated ecosystems , in particular the larger-scale EcoPODs, are of use to as much of the BER community as possible, the workshop participants made the following recommendations:

1. Produce publicly available datasets for a set of control variables. Use for benchmarking, and assessing reproducibility between systems.

2. Implement data standards.

3. Develop/leverage nano/micro sensors and in situ root imaging.

4. Encourage the development of interdisciplinary teams to develop EcoPOD experiments.

5. Develop fabricated ecosystems with size and complexity that sits between the EcoFAB and EcoPOD to accelerate use and access.

INTRODUCTION

Microbes, plants, and their associated ecosystems are critical to many Earth systems processes, including carbon cycling, soil health and water resilience. Practically, the interactions between plants, microbes and their surrounding environment dictate plant productivity, resistance to disease and susceptibility to environmental stressors. Despite the importance of the environment-plant-microbe interface, little is known about the fundamental mechanisms underlying these ecosystem processes. Studies are often undertaken at two very different scales: either highly-replicated systems that can be tested in laboratory settings (culturable microbes, model plants, limited space, short time scales), or field-scale studies (challenging to replicate, reproduce, constrain, manipulate). Hypotheses derived from laboratory-based studies, oftentimes, do not translate into the field, or vice versa.

To bridge this gap, and to explore complex interactions under fully controllable and highly replicated conditions, Berkeley lab is developing a suite of fabricated ecosystems which span spatial and temporal scales — the EcoFAB and the EcoPOD (**Fig 1**). Previous EcoFAB workshop reports can be found **here**. The previous EcoPOD workshop (internal to Berkeley Lab), with report can be found **here**.

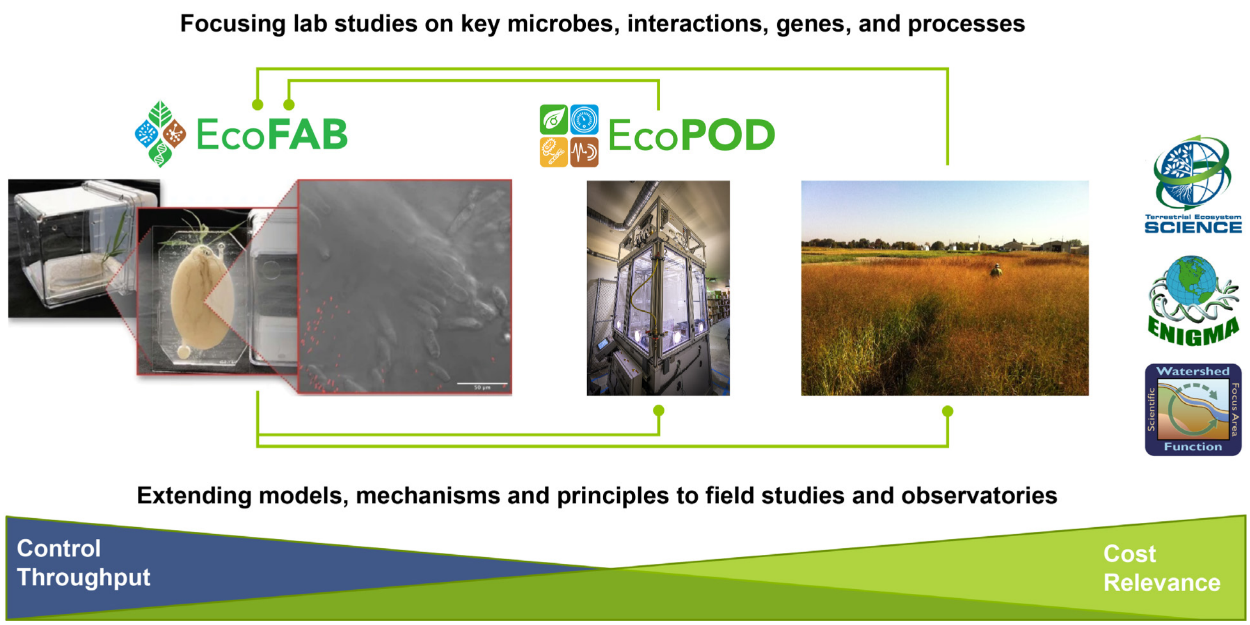

Fig 1: Overview of how fabricated ecosystems can bridge the lab-field scale gap.

EcoFABs: A fabricated ecosystem, or EcoFAB, consists of a physical chamber, components to replicate an ecosystem of interest (e.g., soil, plants), the microbial communities to be tested, and any measurement technologies (e.g., sensors or sampling apparatus) with a focus on plant phenotyping, Omics analysis, and high resolution rhizosphere imaging. This approach can use widely-accessible printing technologies, and most recently injection molding, to fabricate controlled microbiome habitats that can be standardized and easily disseminated between labs.

As an example (**Fig. 2**), researchers at Berkeley Lab use 3D printing to create molds for casting the biocompatible polymer polydimethylsiloxane (PDMS) into the upper portion of a fluidics chamber. This is subsequently attached to a microscope slide, completing the chamber. This is then placed into a sterile container, providing a gnotobiotic system for studying microbial interactions. This chamber design includes a port for growing the model grass *Brachypodium distachyon,* tubing that allows sterile introduction and sampling of microbes and metabolites, and a lighting system. EcoFABs have been tested with different growth media (hydroponics, artificial soil, field soil), different microbes (bacteria, fungi), and plants (Brachypodium, Arabidopsis, switchgrass etc). Data collected in duplicated systems in different labs had excellent reproducibility (**Sasse et al. 2018**).

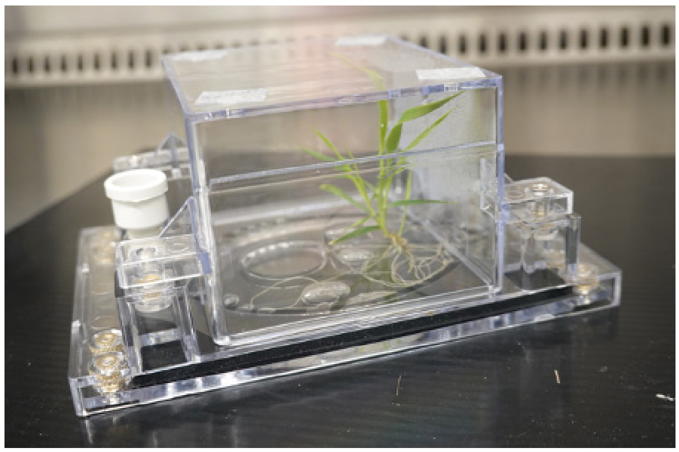

Fig 2: EcoFAB showing *Brachypodium distachyon* growing in hydroponic solution. Image credit: Kateryna Zhalnina.

EcoPODs: An EcoPOD is a larger-scale fabricated ecosystem that allows direct and intensive monitoring and manipulation of replicated plant-soil-microbe-atmosphere interactions over the complete plant life cycle. The EcoPODs will be equipped with environmental controls to carefully manipulate and control temperature, humidity, and other important climatic parameters both above and below ground. The EcoPODs will also be outfitted with sensors capable of monitoring soil moisture, oxygen, and specific nutrients, and the output from these sensors will be integrated using computer models to gain a coherent understanding of the environment inside the EcoPOD. Multiple EcoPODs will allow scientists to examine the impact of differences in types of soils, microbes and plants on ecosystem interactions. The first prototype EcoPOD will be installed at Berkeley Lab in November 2020, based on a model developed at the German Centre for Integrative Biodiversity Research (**iDIV; Fig. 3**) and manufactured by the German company Umwelt Geräte Technik (UGT). This prototype will be used as a base-case for the development of further sensors and imaging capabilities. A vision for 16 EcoPODs, to be placed in the BioEPIC building at Berkeley Lab, is being developed; with a full complement of EcoPODS up to 4 binary variables can be investigated at one time (using field plot designs as inspiration for the experimental design. These will be deployed in collaboration with scientists from academia, national labs, and industry to answer questions that are critical to BER mission.

We propose that there is a wider opportunity to create a network of test-beds of plant-soil-microbial laboratory ecosystems spanning science and security labs. These test-beds will be critical infrastructure in securing natural and agricultural systems, as well as the fundamental science underlying complex ecosystems such as the rhizosphere. This network could span scale, complexity, and sensor deployment. However, we recognise that there are many technical challenges involved in replicating ecosystems, including features that may never be possible to fully recapitulate.

Fig 3: EcoPOD I unit installed at Berkeley Lab. Image credit: Thor Swift/Berkeley Lab.

Thus, as Berkeley Lab continues to develop the EcoFAB, and begins to prototype the EcoPOD, we want to explore both how these developing resources can be leveraged by the larger scientific network; and how they can be integrated with existing and future resources at other national laboratories. This short workshop (2 hour) was composed of a brief introduction, followed by breakout into three groups (**see Appendix 1 for the full agenda**). By holding it at the Genomic Sciences PI meeting (GSP) between sessions, we aimed to capture broad perspectives from across the Biological and Environmental Science (BER) Biological Systems Science (BSS)/Bioenergy Research Center (BRC) portfolio. Each group (**see Appendix 2 for a list of participants**) discussed three different topics, guided by facilitators, and recorded by note-takers: (**1**) Science that can be enabled by fabricated mesocosms; (**2**) Defining the key technological needs for these systems (**3**) How to use fabricated mesocosms to bridge the scale gap between lab and field. We encouraged broad discussion including criticism. The charge questions which were provided to all participants are included in **Appendix 3.**

WORKSHOP FINDINGS

Discussions at the workshop included identifying which scientific questions would benefit from these technologies, challenges in implementing the experiments, benefits of de-risking experiments at smaller scales prior to testing in more complex experimental systems, missing capabilities, as well as the experimental limitations of fabricated ecosystems. Additionally, there was extensive discussion about data standards and sharing. Finally, we discussed approaches for steering and community access.

Reproducibility, Replication, and Limitations

Feedback in this section led to two primary recommendations to facilitate system benchmarking: (1) Work to integrate with NMDC to ensure data collection in fabricated ecosystems is FAIR and compatible between researchers, (2) A thorough series of EcoPOD projects to generate valuable datasets to assess system reproducibility

Fabricated ecosystems provide both an opportunity to bring the field into the lab, and to increase the complexity of typical lab experiments. Critical to the success of these systems is reproducibility and replicability. The EcoFAB ring trial (**1**) is an excellent example of collecting these data so that the community can see how the system performs. EcoFAB designs are disseminated here: **http://eco-fab.org/device-design/** and data is released through the **JGI Genome Portal**. Given ongoing EcoFAB efforts and publications e.g., (**2**) we are very enthusiastic about the developing National Microbiome Data Collaborative (NMDC) capabilities (**https://microbiomedata.org/about/**) and envision that this will be the future home of EcoFAB data. As discussed below, data standards are key to this.

EcoPODs present a larger challenge to understand reproducibility, due to the complexity of the system. EcoPODs are lower-throughput/lower-replication and it would help future users design and plan their experiments if data on reproducibility was available. In addition, some concerns about the reproducibility or relevance of past Ecotron-like experiments were raised. Therefore, it was suggested that a set of EcoPOD experiments are performed which show the extent of reproducibility of data collected. This would include experiments which test reproducibility within one unit between lysimeters (e.g., soil moisture gradient dynamics during drought and corresponding plant growth). These would test single variables and would be paired with field work. One such project is currently underway funded by Berkeley Lab internal research funding (LDRD, PI E. Singer), but further investment in this type of project was considered highly beneficial.

These experiments could also provide valuable benchmarking datasets which can be used as references for field work. For example, iron-deficient experiments in the EcoPOD would generate sets of signature plant and microbial transcripts and metabolites that can be used to survey for iron deficiency in the field. Because of issues of bioavailability, soil micronutrient concentrations are poor indicators of nutrient sufficiency, but these signatures would provide insights into what each organism is experiencing.

Finally, these experiments would act as a pilot for developing data standards and collaborating with system modelers and field researchers, to ensure that data sets that are collected both in fabricated ecosystems and at field sites are

Modeling and Data Standards

Feedback in this section led to the primary recommendation that funding should be prioritized for interdisciplinary teams that combine lab and field based researchers, as well as modelers, to maximise the potential of fabricated ecosystems.

Modeling: One of the key drivers behind developing the fabricated ecosystem infrastructure at Berkeley Lab was to provide a platform for computational modelers to work with experimental biologists to improve our understanding of these complex ecologies. There is a strong scientific need to try to reconcile models driven by lab data and those driven by field data. By identifying which variables are driving the discrepancies between the field and lab data, it will help to prioritize research to understand why we are not able to accurately describe these processes.

As fabricated ecosystems move to more complex microbial communities, the combinatorial possibilities will preclude investigating all possible communities within EcoFABs. Therefore it is critical to make effective use of simulations to predict the most informative community structures. KBase capabilities in flux balance modeling will be central to construction of community models to predict microbial interactions and activities.

In particular, it was clear that fabricated ecosystems would be valuable for improving our understanding of the role that heterogeneity has on models. Effects seen in the lab are often stronger than those observed in the field e.g., nutrient responses due to masking from heterogeneity. For example, how "strong" does an effect in the lab have to be to be important in the field. Can we predict this? In addition, the flow of lab to EcoFAB to EcoPOD and field give some unique opportunities to consider the challenges of modeling across scales using data from the different systems.

Discussions also emphasized that modelers with experience working with experimental data should be included in experimental teams from the beginning, so that the modeling work is fully integrated with the proposed work. In addition, teams should also include those with experience of collecting and working with field data. This will enable the scientists to learn to speak the same language from the start, as well as to design experiments that inform different aspects of plant-microbial-soil science. The Berkeley Lab team are encouraged to help facilitate these interactions, and funding to support these interdisciplinary teams is considered a priority.

Data Standards: Experiments using the EcoPODs will control a number of environmental parameters, including light intensity, relative humidity, above and below ground temperature, soil moisture and, potentially, gas concentrations. Sensors used to control these parameters, as well as measures of plant and microbial response to these conditions have the potential to generate terabytes of information for each replication of each experiment. Many of the comments centered on what to do with this flood of data and how to ensure that it was stored in a way to make it fully accessible and understandable to future researchers.

There was significant discussion on the best way to proceed for standardization of data. There are already a number of ecology community data and metadata standardization groups out there that are working in this area. Two of the leaders in this area are the National Microbiome Data Collaborative (NMDC) and microBEnet for metadata standards. It is important to communicate between different groups, such as experimentalists and modelers, so that the collected data will be useful to the larger scientific community. Additionally, some coordination between different experimental disciplines will be necessary.

Some data-intensive applications have the potential to create computational bottlenecks. Examples given included multispectral imaging of root architecture and next-gen sequencing outputs. Can we leverage efforts by other groups that are working in this area, such as ARPA-E, to manage data on this scale? We will also need to develop more efficient ways of sharing these datasets with other members of the scientific community.

Sensing Capabilities

Feedback in this section led to two primary recommendations: (1) a focus on developing/implementing below ground spatial imaging of the roots and (2) develop/implement miniaturized sensors to maximize feedback in the systems.

Below Ground Sensing: Below ground sensing in the field is a major challenge due to access. The EcoFABs have the advantage of allowing visual access to the root system including use of optodes for monitoring pH, CO_2, O_2, and etc throughout the experimental time course. This allows the user to gather both spatial and temporal information of both root,microbe, and chemistry, as well as any introduced perturbation such as nutrient source. However, the spatial scale is necessarily limited. This is particularly challenging when looking at interactions that take weeks to develop, such as between roots and mycorrhizal fungi, or using larger plant species, such as sorghum. Additionally, there are concerns about the effect of light on the below-group ecosystem which is being addressed by using plastics for the root chamber that don't transmit light in the range sensed by roots. Future challenges include developing miniaturized sensors, e.g., for soil matrix moisture, that are compatible with the EcoFAB.

The EcoPODs have the advantage of being much larger, but with necessarily less visual access to the below ground processes. The capture of the growing root architecture in 3D continues to be a challenge. Developing detailed spatial below-ground information about both plant and microbial location was considered to be both a priority and a major hurdle by many attendees. Current approaches include infrared, X-ray, chemical sensing, and acoustic imaging although all have limitations. The EcoPOD provides an opportunity to collaborate with teams who are developing these methods, such as those at the Danforth Center and the ARPA-E TERRA program, to test existing methods over a range of soil types and conditions such as temperature and soil saturation, as well as develop new methods.

The EcoPOD user has access to the full depth of the soil column. This gives an opportunity to place sensors through the column. Many sensors exist, but may benefit from miniaturization, as space is still relatively limited in the EcoPOD quadrants. This is a good opportunity to develop collaborations with both academia and industry partners for the engineering expertise needed to miniaturize sensors.

Above Ground Sensing: The group noted that above ground phenotyping and sensing is relatively advanced, primarily due to the physical ease of access. As a result, there are many more field-derived datasets to compare to experiments performed in the EcoPOD. This will be useful for benchmarking. In particular, multiple participants raised concerns about whether the artificial lighting was sufficient. Previous Ecotron efforts have often been criticized for the poor quality lighting but recent advances in LED lighting (including the lights proposed for installation in the first EcoPOD prototype at Berkeley Lab) may resolve much of this and the EcoFAB team has recently found that they can simulate field lighting conditions using new PHYTOFY RL LED lights which are tunable in 6 different spectra from UVA to far red that could also be used in EcoPODs. Collecting sensor and imaging data on aerial growth of plants under a set of standard conditions in a defined field soil should be a priority.

There has been much recent interest in predictive phenotyping, where data on young plants is predictive of mature traits such as yield and grain quality. This has been developed in field systems, including via Advanced Research Projects Agency–Energy (ARPA-E) funded projects such as Transportation Energy Resources from Renewable Agriculture (TERRA), but the EcoPOD would allow further interrogation of these models, in particular the response to individual climatic parameters e.g., drought vs. temperature.

Scientific Opportunities

Feedback in this section is that there are a vast range of potential scientific questions that can be answered with these systems, and the primary recommendation was that experimental setups that allow multiple questions to be answered during one "run" should be prioritized.

The addition of EcoPOD capabilities to bridge the gap between small-scale EcoPOD and large-scale field capabilities will enable scientists to address critical DOE missions in energy and the environment. For example these capabilities will enable elucidation of molecular mechanisms by which microbial communities and abiotic constraints control key geochemical processes such as soil carbon cycling. They will also enable rapid development and translation of beneficial microbial communities from benchtop to field applications to support efforts in sustainable bioenergy and bioproducts. Importantly, the containment and control afforded by EcoPODs and EcoFABs will enable pioneering studies in secure biosystems design to provide key insights into the persistence, fate, and control of engineered microorganisms within soil microenvironments. Finally, there are opportunities to collaborate with other fabricated ecosystem projects at various scales across the globe (**3**).

Biosecurity Experimental Opportunities: There was a lot of enthusiasm from the participants about using the EcoPODs for experiments that are challenging to do in the field due to regulatory or safety concerns. Examples include understanding the persistence and fate of engineered microbes within contained and controlled environments to identify risks and effective containment strategies. It was also noted that it's an opportunity to monitor the effects of potentially environmentally hazardous materials such as plastic microfibers or carbon nanotubes. There was also interest from National Labs with secure facilities who could adapt technologies such as the EcoBOT, a robot that enables automated EcoFAB experiments, and EcoPOD, once they are derisked and developed further, for work which requires secure facilities that are not available at Berkeley Lab (such as at Sandia and Los Alamos).

Fundamental Science Opportunities: Due to the breadth of scientific background among our workshop participants, many great experimental ideas were discussed including topics such as carbon sequestration experiments, for which the EcoPOD's semi-closed system can facilitate mass balance calculations easier than in field experiments. Furthermore, deep soil processes were discussed as most data from soil experiments does not exceed the top 10 cm. Additionally, soil-atmosphere gas exchange was mentioned, which we will keep in mind as we are aiming to develop future prototypes that can provide gas-tight conditions. Similarly, climate change simulations that include warming or elevated CO_2 concentrations are a future experimental goal that will require prototype updates that are achievable. All participants were excited about the possibility of separating individual environmental parameters to observe their effect on the plant-microbe-soil-atmosphere ecosystem. This includes being able to better predict the phenotype of engineered bioenergy crops under different environmental conditions, reducing the need for expensive and complex multi-site field trials.

Access and Steering

Feedback in this section led to the recommendation of the formation of a steering committee for the EcoPODs, similar to that formed for the EcoFAB program, which has proved to be successful in developing the EcoFAB as an accessible platform.

The EcoFAB team has made access to the units purchased by Berkeley Lab a priority. For example, detailed protocols on how to fabricate them have been published (**4**), and TEAMS, a project funded by DOE, is dedicated towards the dissemination of EcoFAB supplies and protocols including model microbiomes to foster interlaboratory science and experimental standards. Through these efforts, laboratories across the globe are now using Berkeley Lab-designed EcoFABs as well as developing their own iterations, with a new EcoFAB ring-trial study with *B. distachyon* and a synthetic microbial community (SynCom) planned for early 2021.

As discussed above, EcoFABs and EcoPODs are complementary technologies with different strengths and weaknesses. One way Berkeley Lab is leveraging this fact is through the use of higher throughput EcoFABs experiments to assess important parameters that can later be implemented within EcoPODs. The EcoBOT being developed at Berkeley Lab through the Trial Ecosystems for the Advancement of Microbiome Science (**TEAMS**) project will support remote, high throughput EcoFAB experiments to improve to improve turnaround, standardization, and reproducibility for EcoFAB experiments (**Fig 4**).

Fig 4: EcoBOT TEAMS — left photo shows the EcoBOT system, right photo shows how the EcoFABs can be arrayed within the EcoBOT.

Participants were enthusiastic about collaborating with Berkeley Lab scientists to make use of EcoPODs, but concerns were also raised about limited access due to throughput. It was suggested that this could be addressed in a number of ways:

1. Assembly of a steering committee to advise on long-term development goals and help to select collaborators. It was suggested that committee members should consist of leaders in their respective scientific fields of research and should have a strong interest in the continued success of the program. Representation will include scientists from other National Laboratories as well as from relevant universities. Subject matter experts would potentially include plant and microbial ecologists with emphasis in carbon cycling or agriculture, computational biologists with emphasis in climate studies or modelling, and physical-chemical scientists with emphasis in plant and microbial metabolites.
2. The Berkeley Lab EcoPOD team could advise and collaborate with other institutions who are interested in applying for funding to purchase and host their own EcoPODs. This would not be limited to DOE-BER funds, and examples suggested include the NSF Major Research Instrumentation program.
3. Develop fabricated ecosystems with size and complexity that sits between the EcoFAB and EcoPOD to accelerate use and access. These would have a smaller footprint, cost less, and could have increased replication. These could make use of the EcoPOD infrastructure, instrumentation, and data handling, but be designed to answer a smaller set of scientific questions. For example, questions that do not involve plants only require the lower part of the unit, whereas questions around aerial plant development may not require below ground sensors. Development could also be done in collaboration with industry partners, as simpler units may be of use to the agbiotech industry.
4. Build strong relationships with related programs which cover scales outside of those planned at Berkeley Lab, such as LEO (Landscape Evolution Observatory), and with other agencies who fund relevant research (National Science Foundation, US Department of Agriculture, National Aeronautics and Space Administration). In addition, there is expertise at other national laboratories e.g., geospatial modeling which should be sought out as part of developing new projects.

CONCLUSIONS AND NEXT STEPS

The plant-soil-microbe interface is immensely complex, but understanding is critical as humanity faces challenges which include accurately predicting the effects of rising atmospheric CO_2 on ecosystem dynamics, to sustainably producing the biomass needed for a thriving bioeconomy. Fabricated ecosystems are a promising tool to link research efforts in the lab and field. The following next steps were suggested:

- Interdisciplinary teams should be supported, which specifically include modelers.
- New intermediate scale/simpler designs should be supported — potentially collaborate with other institutions to site them.
- Reproducibility at all scales needs to be tested and reported on.
- Scientific questions with a focus on biosecurity questions should be prioritized.
- Data management, standards and sharing should be a priority.
- Scientific questions should be tested in parallel with sensor development projects.
- A broad steering committee should be assembled.

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APPENDIX 1: LIST OF PARTICIPANTS

Participants	Affiliation
Jenny Mortimer	Berkeley Lab
Trent Northen	Berkeley Lab
Esther Singer	Berkeley Lab
Costas Maranas	Penn State
Hector Garcia Martin	Berkeley Lab
Dan Jacobsen	Oak Ridge National Laboratory
Sue Rhee	Stanford
Steve Allison	UC Irvine
Claudia Weihe	UC Irvine
Daniel Segre	Boston University
Mitch Doktycz	Oak Ridge National Laboratory
Dale Pelletier	Oak Ridge National Laboratory
Melissa Cregger	Oak Ridge National Laboratory
Janet Jansson	Pacific Northwest National Laboratory
Federica Brandizzi	Michigan State University
Aymerick Eudes	Berkeley Lab
John Dunbar	Lawrence Livermore National Laboratory
Mary Firestone	UC Berkeley
Umakant Mishra	Argonne National Laboratory
Kirsten Hofmockel	Pacific Northwest National Laboratory
Patrick Chain	Los Alamos National Laboratory
Cristal Zuniga	UC San Diego
Erin Nuccio	Lawrence Livermore National Laboratory
Sanna Sevanto	Los Alamos National Laboratory
Louise Glass	UC Berkeley/Berkeley Lab
Jessy Labbe	Oak Ridge National Laboratory
Kate Zhalnina	Berkeley Lab
Crysten Blaby-Haas	Brookhaven National Laboratory
Dan Noguera	UW/GLBRC
Adam Deutschbauer	Berkeley Lab
Tom Juenger	UT Austin
Romy Chakraborty	Berkeley Lab
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Henrik Scheller	UC Berkeley/Berkeley Lab
Javier Ceja Navarro	Berkeley Lab
Kristen DeAngelis	University of Massachusetts, Amherst
Chuck Smallwood	Sandia

APPENDIX 2: WORKSHOP AGENDA

January 25, 2020

Time	Topic	Speaker/Discussion leader
12:00–12:15 pm	**Welcome and introductions**	Jenny Mortimer/Trent Northen
12:15–12:30 pm	**Overview of EcoPODs and workshop**	Jenny Mortimer
12:30–12:50 pm	**Breakout round 1**	all
12:50–1:10 pm	**Breakout round 2**	all
1:10–1:30 pm	**Breakout round 3**	all
1:30–1:35 pm	**regroup**	all
1:35–1:45 pm	**Breakout summaries**	Table facilitators (assisted by notetakers)
1:45–2:00 pm	**Wrap up and next steps**	Jenny Mortimer

APPENDIX 3: MATERIALS PROVIDED TO PARTICIPANTS IN ADVANCE

Preparation for Fabricated Mesocosm Workshop

Monday 24th February 2020, 12:00–2:00 pm, GSP meeting

Previous EcoFAB workshop reports can be found **here**. The previous EcoPOD workshop, with report can be found **here**. The agenda is appended at the end of this document.

Ecosystem fabrication is an approach to creating controlled microbial, soil and plant ecologies within a laboratory setting that enable discovery and dissection of environmental variables, activities, and interactions. At Berkeley Lab, we are developing two systems which span spatial and temporal scales — the **EcoFAB** and the **EcoPOD.**

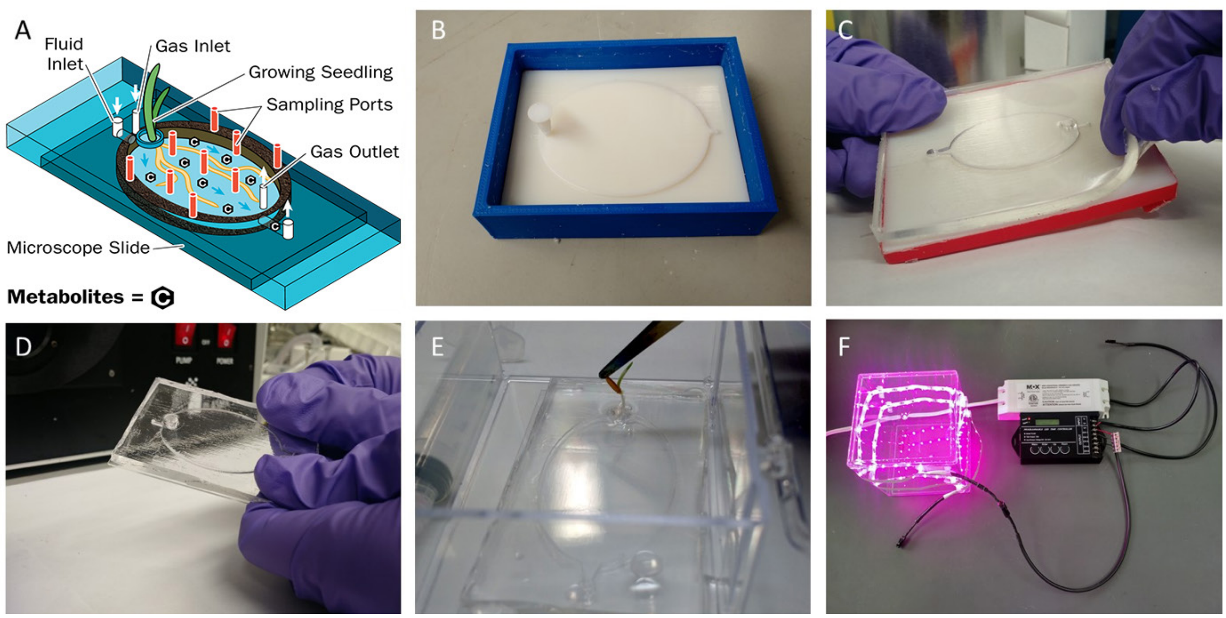

Fig. 5: EcoFAB fabrication and use (from EcoFAB summit report, 2017).

EcoFABs: A fabricated ecosystem, or EcoFAB, consists of a physical chamber, components to replicate an ecosystem of interest (e.g., soil, plants), the microbial communities to be tested, and any measurement technologies (e.g., sensors or sampling apparatus). This approach uses widely-accessible 3D printing technologies to fabricate controlled microbiome habitats that can be standardized and easily disseminated between labs. As an example (**Fig. 5**), researchers at Berkeley Lab use 3D printing to create molds for casting the biocompatible polymer polydimethylsiloxane (PDMS) into the upper portion of a fluidics chamber. This is subsequently attached to a microscope slide, completing the chamber. This is then placed into a sterile container, providing a gnotobiotic system for studying microbial interactions. This chamber design includes a port for growing the model grass *Brachypodium distachyon,* tubing that allows sterile introduction and sampling of microbes and metabolites, and a lighting system. EcoFABs have been tested with different growth media (hydroponics, artificial soil, field soil), different microbes (bacteria,fungi), and plants (Brachypodium, Arabidopsis, switchgrass etc). Data collected in duplicated systems in different labs had excellent reproducibility (Sasse et al. 2018).

EcoPODs: An EcoPOD is a larger-scale fabricated ecosystem that allows direct and intensive monitoring and manipulation of replicated plant-soil-microbe-atmosphere interactions over the complete plant life cycle. The EcoPODs will be equipped with environmental controls to carefully manipulate and control temperature, humidity, and other important climatic parameters both above and below ground. The EcoPODs will also be outfitted with sensors capable of monitoring soil moisture, oxygen, and specific nutrients, and the output from these sensors will be integrated using computer models to gain a coherent understanding of the environment inside the EcoPOD. Multiple EcoPODs will allow scientists to examine the impact of differences in types of soils, microbes and plants on ecosystem interactions. The first prototype EcoPOD will be installed at Berkeley Lab in March 2020, based on a model developed at the German Centre for Integrative Biodiversity Research (**iDIV**; **Fig. 6**). This prototype will be used as a base-case for the development of further sensors and imaging capabilities. A vision for 16 EcoPODs, to be placed in the BioEPIC building at Berkeley Lab, is being developed; with a full complement of EcoPODS up to 4 binary variables can be investigated at one time (using field plot designs as inspiration for the experimental design. These will be deployed in collaboration with scientists from academia, national labs, and industry to answer questions that are critical to BER mission.

Fig 6: iDIV Ecotrons in Leipzig and at Umwelt-Geraete-Technik GmbH.

The Goal of this Workshop: We propose that there is a wider opportunity to create a network of test-beds of plant-soil-microbial laboratory ecosystems spanning science and security labs. These test-beds will be critical infrastructure in securing natural and agricultural systems, as well as the fundamental science underlying complex ecosystems such as the rhizosphere. This network could span scale, complexity, and sensor deployment. However, we recognise that there are many technical challenges involved in replicating ecosystems, including features that it may never be possible to fully capture.

As we continue to develop the EcoFAB, and begin to prototype the EcoPOD, we want to explore:

1. How these developing resources can be leveraged by the larger scientific network.
2. How they can be integrated with existing and future resources at other national laboratories.

In this workshop, there will be 3 discussion sessions, led by facilitators, followed by a report out at the end. Everyone will have a chance to engage with all 3 topics. The topics for each session along with some discussion questions are provided below, but we are looking for a broad discussion, including criticisms.

Possible Use Cases

Climate Resilience: There is broad agreement that the US will experience increased extreme weather and dramatically altered rainfall. Yet, it is uncertain how plants will respond Microbiomes have been shown to reduce diverse climate associated stresses from drought, extreme temperatures. Central to these processes are the production of soil organic matter that retains water, nutrients, and improves water infiltration. Fabricated ecosystems can be used as a complimentary strategy to field research to explore these processes over smaller spatial and temporal scales. The ability to replicate the system, to control single variables, and to use engineered organisms (including biosensors) will enable both measurement and perturbation.

Biosecurity: Advances in biological engineering technology, climate change, and the speed of global movement have all raised the threat of biosecurity, whether the release origin is unintentional or malevolent. Critical to mitigating these threats is the rapid and sensitive biosurveillance. Developing and validating detection technologies will require contained ecosystems. Use cases include establishment of ecosystem baselines, and development of new remote/unmonitored detection technologies. They can also operate as testbeds for the validation of novel detection technologies (both computational, and hardware). By operating across scales and complexities (EcoFAB, EcoPOD and beyond to e.g., **EcoCELLs**), systems can be developed with the appropriate sensors and containment.

Development and testing models of dynamic biological processes: A mesocosm scale environment provides an opportunity to uncover plant-microbe relationships to inform models that cannot be addressed at the laboratory or greenhouse container scale. We can exploit heterogeneity within an EcoPOD environment to achieve more replication with increased sampling sites to test for causal aspects of heterogeneity to better understand multi-trophic interactions. As it is difficult to identify all variables that may occur at field scale, it will be important to develop reduced order surrogate models. The EcoPOD scale will help in determining what portions of the simulation must be exact and what portion can be approximated. This could lead to efforts to develop a “digital twin” or digital replica of a functional ecological model in the EcoPOD, making use of the real-time data available from the sensors.

Breakout Session Topics

Everyone has been assigned to a group (1, 2, or 3). Please gather in one area of the room as directed, and stay there throughout. The facilitators/note takers will rotate so that you will have a chance to discuss each topic during the course event.

The GoogleDoc containing the notes can be accessed **here**. Please feel free to add notes during the session, in addition to those taken by the facilitator/note-taker of each group, as well as before or after the workshop. We want to get as many views as possible. Thanks! Any issues with access, please contact Jenny Mortimer (jcmortimer@lbl.gov).

Topic 1

Esther Singer, Facilitator
Louise Glass, Note-taker

What type of experiments could you only do in the EcoPods? How would they complement your existing or future capabilities and expertise? How would this enable new research directions?

Topic 2

Hector Garcia, Martin, Facilitator
Aymerick Eudes, Note taker

What are the key technological needs for these systems (both hardware and software)?

Topic 3

Kate Zhalnina, Facilitator
Trent Northen, Note taker

How do we connect platforms across scales and complexity (e.g., EcoFab to fields) in experiments? How do we integrate and generalize our findings? What are the highest impactful scientific challenges that we should focus on?