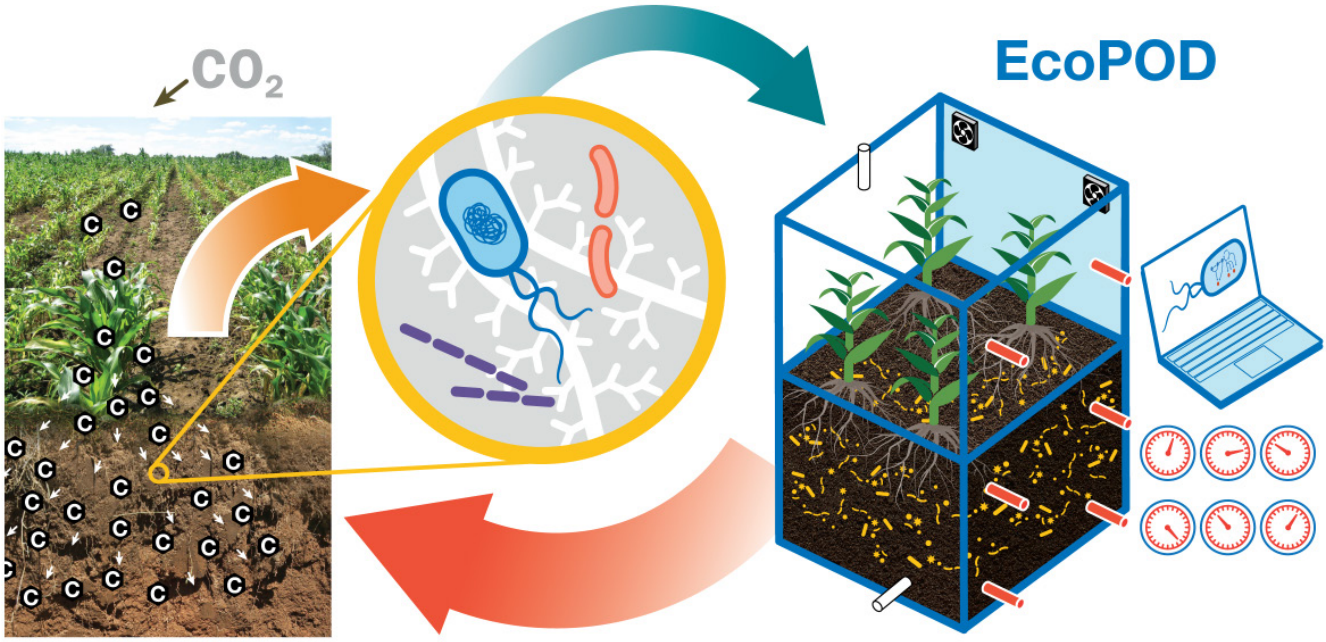
EcoPOD Workshop

EGSB
Environmental Genomics & Systems Biology

Bridging Laboratory to Field Science Using Synthetic Mesocosms



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Organizers

Jenny Mortimer, Ph.D.
Environmental Genomics and Systems Biology (EGSB)
jcmortimer@lbl.gov
510-486-6627

Gary Andersen, Ph.D.
Environmental Genomics and Systems Biology
glandersen@lbl.gov
510-495-2795

Report prepared by: Jenny Mortimer, Gary Andersen, N. Louise Glass (EGSB), and Katy Christiansen (Biosciences Area Office), with additional input from Esther Singer (JGI) and Romy Chakraborty (Earth & Environmental Sciences Area, EESA).

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Executive Summary

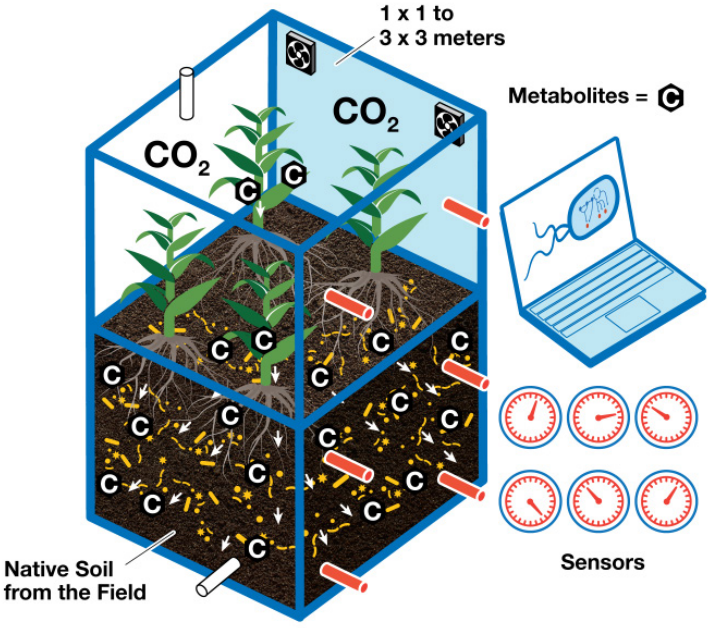
To meet growing agricultural demands for food and energy production, as well as to reduce loss of carbon from soils, it is necessary to understand how to sustainably maintain healthy soils and restore unhealthy ones. Current studies are limited by the use of low complexity, greatly simplified laboratory studies and high-complexity, challenging to reproduce field studies. We envision that an improved generalized understanding of ecosystems can be addressed by developing highly instrumented, synthetic, replicated ecosystems.

Berkeley Lab is developing a research program focused on the development and use of EcoPODs (Figure 1), ecosystem manipulatories that can replicate natural ecosystems in a laboratory setting. EcoPODs are enclosed mesocosm-scale ecosystems that will enable the control, manipulation, and measurement of more complex ecosystems and soil monoliths that can be reiteratively interrogated in concert with experiments conducted across scales. EcoPODs allow longer-term experiments to be performed, enabling replicated temporal and spatial investigations.

The EcoPOD Program leverages Berkeley Lab's world-leading capabilities in plant, microbial & environmental science, computation, modeling, and engineering to develop laboratory-based infrastructure that can mimic aspects of field research sites. We anticipate that prototype EcoPODs will range from a 1–3 m^3 closed environment that will allow for direct and intensive monitoring and manipulation of replicated plant-soil-microbe-atmosphere interactions over relevant time scales. These "pilot-scale" ecosystems will be equipped with environmental controls to carefully manipulate and control temperature, humidity, and other important climatic parameters. An important aspect of EcoPOD development is the need for state-of-the-art technologies for precise, fine-scale environmental sensors as well as new capabilities for real-time imaging, sample collection and data integration.

To inform Berkeley Lab's EcoPOD development, an internal workshop was convened to introduce the EcoPOD concept to the wider lab community and to gather input on EcoPOD development requirements and scientific use cases for EcoPODs. The workshop participants identified basic requirements for building EcoPODs, the scientific gaps that can be addressed by EcoPODs, and possible experiments to prove out these systems.

Figure 1: An overview of the EcoPOD concept



Introduction

Defining the drivers of ecosystem function is an important endeavor to understand natural systems, which may be experiencing altered climate, as well as for managed agricultural and grassland ecosystems. A vital aspect of soil health is the accessibility of carbon, which is critical to soil function and productivity of plants. Increased soil organic carbon means a more stable structure, resulting in better aeration and drainage, improved ability to hold water near plant roots, and reduced erosion and nutrient leaching. Conventional tillage and crop harvesting methods disrupt natural carbon storage, releasing over 80 billion tons of stored soil carbon as CO_2 annually into the atmosphere. While current farming practices have fed a growing global population, farmers have relied on fertilization with inorganic nutrients that are expensive and in short supply to increase crop productivity. Management practices such as no-till farming, planting of cover crops, or organic soil amendments are helpful, but *sustainable* strategies to restore and maintain healthy soils long term are urgently needed. Capturing and storing atmospheric carbon belowground and thereby rejuvenating depleted soils will be critical components of sustainable ecosystem function in the future. Achieving this goal will require the right combination of plants, associated microbes, water, and other nutrients, and these combinations will need to be customized for different soils and climates. Identifying and *understanding* these combinations requires the development of new technologies to manipulate and measure ecosystems under highly controlled environments.

Many of the challenges listed above have been cited in reports released by the Department of Energy (DOE), including the recently released report from the Office of Science Office of Biological and Environmental Research's Advisory Committee (BERAC), **Grand Challenges for Biological and Environmental Research: Progress and Future Vision**,* which highlights many areas where the use of reproducible, controlled ecosystems can provide insights into key biological and environmental questions. The grand challenges relevant to this type of system include: understanding the biological complexity of plants and microbes across scales, understanding the links between genotype and phenotype in complex biological communities, and the integration of data from diverse assays and analyses to understand and predict both community and ecosystem dynamics. This report also calls for the development and use of technologies for measuring biological processes to gain new insights; development of these controlled ecosystems would directly contribute to this goal. DOE's applied research programs have also recognized the importance of understanding ecosystem processes. DOE's Bioenergy Technologies Office has identified key research needs for development of sustainable bioenergy crops. In a recent report, **Incorporating Bioenergy in Sustainable Landscape Design**,† challenges in understanding tradeoffs between different priorities (crop production, biodiversity, water and nutrient usage, etc.) were identified. While potential solutions included integrating modeling with field experiments, controlled ecosystems could help bridge those studies through hypothesis-driven experimentation in controlled, instrumented systems.

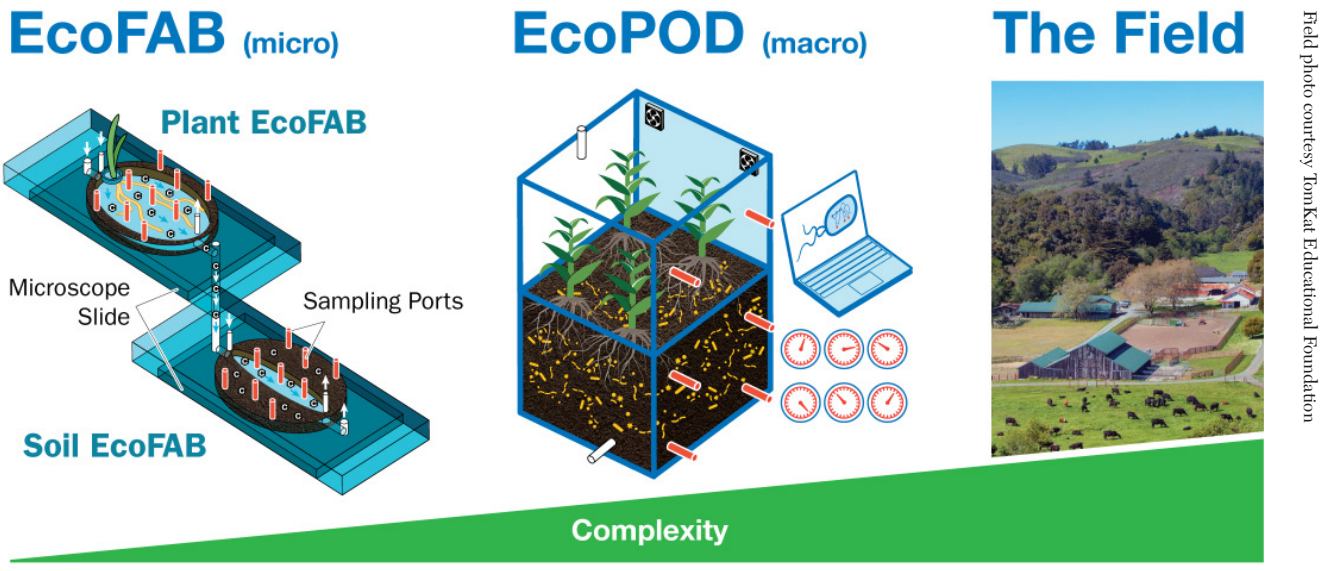
We envision that the process of developing a generalized understanding of ecosystems can be addressed by developing highly instrumented, synthetic, mesocosm-scale ecosystems. Berkeley Lab is already pioneering work in this field through the development of miniaturized EcoFABs (fabricated ecosystems) in a consortium led by the Northen group (**eco-fab.org**). EcoFABs can be considered as part of a continuum of ecosystem manipulatories designed across scales, with

EcoFABs being the smallest format, enabling inexpensive fabrication and distribution across labs (Figure 2). As a next step in the continuum of ecosystem manipulatories, EcoPODs represent a ~1.5 m x 1.5 m x 3 m enclosed ecosystem that will enable the control, manipulation, and measurement of more complex ecosystems and soil monoliths (soil excavated from the environment as an intact block, so as to maintain soil structures) that can be reiteratively interrogated in concert with experiments conducted across scales in EcoFABs and in ecosystems. For example, in contrast to EcoFABs, EcoPODs allow longer-term experiments to be performed, such as analyzing the recruitment and development of a microbiome across a plant's complete life cycle.

The EcoPOD Program will leverage Berkeley Lab's world-leading capabilities in plant & environmental science, computation, modeling, and engineering to develop laboratory-based infrastructure that can mimic aspects of field research sites. We anticipate that prototype EcoPODs will range from a 1–3 cubic meter closed environment that will allow for direct and intensive monitoring and manipulation of replicated plant-soil-microbe-atmosphere interactions over relevant time scales. These "pilot-scale" ecosystems will be equipped with environmental controls to carefully manipulate and control temperature, humidity, and other important climatic parameters. An important aspect of EcoPOD development is the need for state-of-the-art technologies for precise, fine-scale environmental sensors as well as new capabilities for real-time imaging, sample collection, and data integration.

The concept of synthetic ecosystems has not been without controversy, as scientists have argued the extent to which synthetic mesocosm experiments can truly represent ecosystem processes. However, advances in understanding the complexities of the plant-soil microbiome, new measurement techniques, and big-data modeling have driven renewed interest in developing new, highly-instrumented mesocosm systems. Indeed, a number of large scale facilities in Europe have recently been completed or are in process (see summary of current mesocosms below). We believe that Berkeley Lab has unique opportunity to become a leader in the field in development of EcoPOD mesocosms, both in technology development and deployment, and in hypothesis testing of ecological principles. Berkeley Lab is already a world leader in the development of environmental sensors, ecosystem modeling, and plant-microbe-soil research. By leveraging existing work at the laboratory and field scale, the controlled environments of the EcoPODs will allow us to develop a predictive understanding of dynamic biological processes involved in plant productivity and carbon cycling. Examples of how the controlled environment of the EcoPODs could improve current ecosystem models include: understanding the impact of how varying amounts of CO_2 alter carbon flow above and below ground, potential synergistic effects of warming and atmospheric CO_2 concentration, drought impact on the interaction between plants and the rhizosphere microbiome, and understanding and manipulation of trophic networks (plants, fungi, bacteria/archaea, and metazoans).

Figure 2: Different scales for investigating plant-soil-microbe interactions



Field photo courtesy TomKat Educational Foundation

About the Berkeley Lab Internal EcoPOD Workshop

The EcoPOD workshop was held on Thursday June 7th 2018 at the Berkeley Lab Chu Hall facility. Invitations were extended to scientists and engineers across Berkeley Lab, as well as a small number of external participants. Diverse scientific perspectives were represented for this multidisciplinary initiative, 40 participants representing all six research areas at Berkeley Lab attended the workshop (Appendix 3). The EcoPOD workshop had two purposes: to explain the broad goals of the EcoPOD project, and to invite ideas and suggestions to shape infrastructure development and address scientific questions for EcoPOD development and use. As such, the workshop was divided into two parts (Appendix 1). The first half of the workshop consisted of a series of short talks outlining the EcoPOD program at Berkeley Lab, existing global mesocosm infrastructures, and scientific programs at Berkeley Lab that make use or will make use of these controlled ecosystem manipulatories. The second half of the workshop, and the focus of the day, comprised breakout sessions, led by different scientists, and assisted by scribes to capture key outcomes of the discussion. Each discussion leader was provided with charge questions (Appendix 2) to initiate discussion among the participants with diverse backgrounds.

This report summarizes existing global mesocosm programs, provides test science use cases at Berkeley Lab, and summaries of each of the four breakout sessions.

Current Mesocosms

The concept of controlled environments for exploring plant ecophysiology are not new, and have been around since the late 1940s. Frits Went originally developed a Phytotron at Caltech (Munns, 2014), a "Climatron" in Saint Louis, Missouri, and more recently, the EcoPhysiology Lab at the Desert Research Institute (DRI) in Reno Nevada, now the home of the EcoCELLs (see below). Went's work included fundamental research on circadian rhythms, on the effects of air pollution on plant growth, and on the identification of environmental conditions to maximize productivity of many crops. It also inspired the development of the EcoTron program at Centre National de la Recherche Scientifique (CNRS), Montpellier, France (see below), and the Ecotron at Imperial College London, UK. A more detailed history can be found here (**http://www.ecotron.cnrs.fr/index.php/context/historical-perspective**).

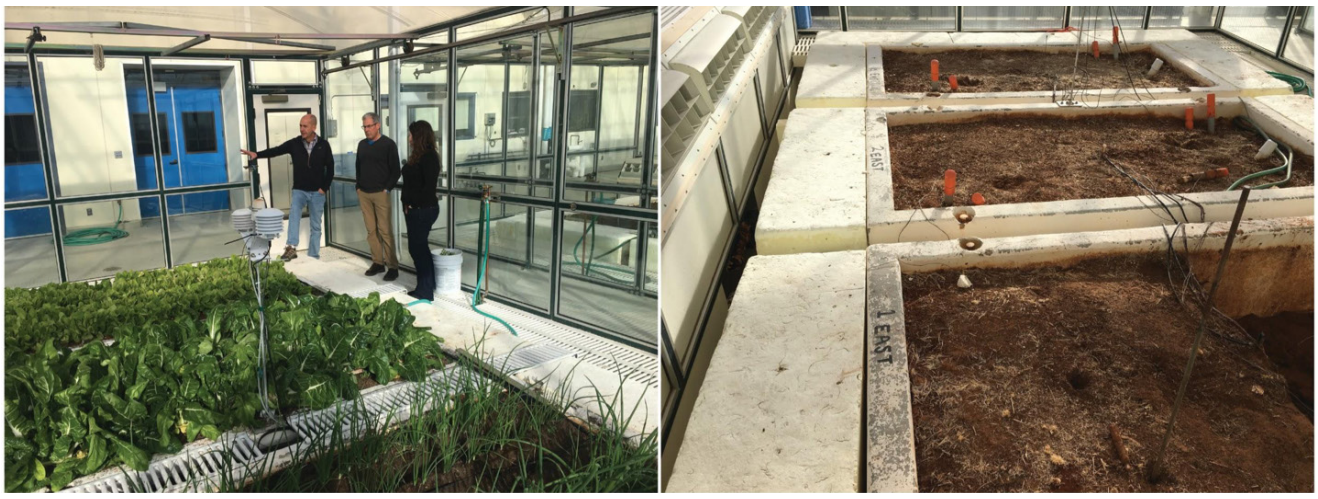


Figure 3: EcoCELLs at the DRI, University of Nevada Reno.

Photo: Gary Andersen

Currently, there are a small number of "next-generation" mesocosms. The LBNL-EcoPOD team has explored these projects, contacted the researchers, and, in some cases visited them, and are now developing collaborations with some of these institutes, described below.

The DRI, part of the University of Nevada, Reno developed the Ecologically Controlled Enclosed Lysimeter Laboratories (EcoCELLs (Griffin et al., 1996) (Arnone et al., 2008)) which is currently led by Drs. Jay Arnone and Richard Jason. There are four EcoCELLs, 184 m^3, each containing three soil monoliths (2.7 x 1.8 x 2 m) in a lysimeter (Figure 3). Within each EcoCELL, the air temperature, humidity, and gas concentrations are measured and controlled. The EcoCELLs were designed for long-term (1–4 years) ecosystem-level experiments. EcoCELLs are much larger/low-throughput than the envisioned LBNL EcoPODs.

The CNRS EcoTron facility in Montpellier, led by Dr. Jacques Roy has led to a revived interest in using controlled environments for studying plant ecophysiology. Arising out of the pioneering work of Dr. Frode Eckardt, the CNRS EcoTron consists of three different experimental scales: Micro-, Meso- and Macrocosms. The twelve Microcosms are 1 m^3, and are growth chambers in which the temperature, humidity, CO_2 and $^{13}CO_2$ can be controlled, and have been operational since 2014. The Mesocosms are currently under development, and include 24 outdoor chambers (1 m^2, 2 m soil depth, 2 m canopy height). The Macroscosms consist of 12 outdoor independent units with a soil depth of up to 2 m, a canopy height of 2 m, and a canopy area of up to 5m^2 (Resco de Dios et al., 2016). As for the EcoCELLS, the environment is tightly controlled and measured, with a focus on gas exchange. Detailed specifications can be found here: **http://ecotron.cnrs.fr/**.

iDIV, the German Center for Integrative Biodiversity Research, is a Deutsche Forschungsgemeinschaft (DFG) funded center and a collaboration between Leipzig University, Martin Luther University Halle-Wittenberg and Friedrich Schiller University Jena. The Ecotron facility is led by Prof. Nico Eisenhauer and Dr. Manfred Türke and consists of 24 enclosed, free-standing units (EcoUnits) situated in a controlled environment (**www.idiv.de**)(Figure 4) designed in collaboration with two companies, one of whom (UGT) is now manufacturing fully customizable versions (**https://www.soilmoisture.com/**). These EcoUnits are fully instrumented and controllable, and a flexible design allows each unit to be split into four sections to increase replication of some variables (Figure 3). Each EcoUnit is 1.55 × 1.55 × 3.20 m, which can be configured either with 1.23 m^3 of soil (~2.2 t) or four cylindrical lysimeters (0.16 m^3 soil). The cylinders can either be used to excavate a monolith or can be hand-filled with sieved soil. iDIV has spent 3 years developing the prototype, and comparing reproducibility between chambers (Eisenhauer & Türke, 2018). The goal of their current research is to explore the effect of changing climatic conditions in southern Germany on native ecosystems.

The EcoTron facilities at Hasselt University, Belgium, is based on the Montpelier Mesocosm design are in the final stages of testing (**https://www.uhasselt.be/UH/FieldResearchCentre/Infrastructure/ECOTRON-Hasselt-University.html**). These units have 2 m diameter x 1.5 m

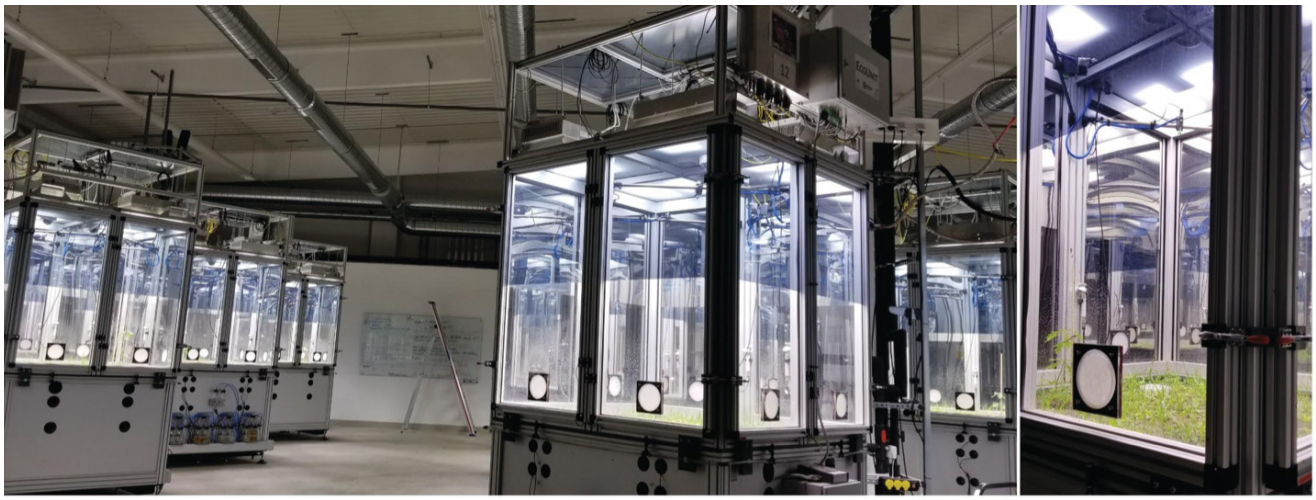


Figure 4: EcoUnits at iDIV, Leipzig. Photo: Jenny Mortimer

height soil monoliths from heathland. Uniquely, these mesocosms are linked to an ecosystem measuring tower (part of the European Integrated Carbon Observation Systems (ICOS)), and will exactly replicate the weather measured at the site from which the monoliths were excavated. The New Zealand Biotron, led by Stu Larsen, explores various conditions under which exotic pests might survive. It consists of six growth chambers, each equipped with a rhizotron room underneath (**http://bioprotection.org.nz/Facilities/new-zealand-biotron/**). The EcoTron Gembloux Agro-Biotech (University of Liege, Belgium) has also begun (May 2018) construction of 10 indoor mesocosms with a canopy height of 1.5 m and 3.1 m^3 soil **http://www.gembloux.ulg.ac.be/environmentislife/ecotron-2/**. Finally, the terraXcube in Bozen, Italy, is designed to simulate extreme environments (**https://terraxcube.eurac.edu/**), and will be used for medical and technological research, as well as ecophysiology.

The development of Berkeley Lab EcoPODs will support a range of science, spanning ecologists to molecular biologists to sensor developers, to engineers and computational modelers. We envision an infrastructure of indoor, stand-alone units, inspired by those at iDIV. These EcoPOD units will need to be flexible and highly customized for our requirements. We are currently developing a prototype for testing, prior to developing the full Berkeley EcoPOD facility.

The advent of new technologies and data generation in natural and managed ecosystems, such as hyperspectral imaging, microbial metagenome sequencing, sensitive sensors for plant photosynthetic capacity and volatile production, and metabolic modeling have provided exciting new hypotheses regarding ecosystem functions. We strongly believe that there is a national need for the development of highly reproducible ecosystem manipulatories that can directly test these hypotheses by manipulating plant genotype, microbial communities and genotypes, trophic interactions, and environmental and soil parameters. We believe that the Berkeley Lab is uniquely aligned to lead in the development of these technologies, especially in the areas of new sensor technologies, sampling technologies and data integration, and analyses needed to interpret experimental variables and results. The development of fully functional EcoPOD units will not only enable experimentation at the National Labs, but will enable research at academic institutions and in biotechnology and agricultural industries interested, for example, in testing seed/soil microbial amendments, drought stress (see below), and other agriculturally-relevant traits.

Use Cases

1 Drought Tolerance in Grasses

Esther Singer, a project scientist at Berkeley Lab was recently awarded an internal Early Career (EC) award through Berkeley Lab's Laboratory Directed Research and Development (LDRD) funding program. The EC LDRD will use EcoPODs to calibrate the first prototype against the 'real world' (UT Austin field site; Figure 5), to study drought stress in *Panicum hallii*, a bioenergy and drought tolerance model, and to compare results between EcoPODs and conventional growth chambers and greenhouses. The idea is to generate a benchmark dataset that allows side-by-side comparison of a range of metrics measured in the EcoPODs and in the field using the same plant genotypes, soils and microbial communities. During the course of the LDRD project, her goals include the development and application of EcoPOD sensors to generate multi-faceted datasets that include plant characteristics, microbial community analytics, and measurements of headspace as well as soil parameters. The biotic components will provide information in the form of plant and microbial abundance/biomass, DNA, RNA, protein, and metabolites that can be used to enumerate, identify, classify, and track growth of microbial and metabolite diversity over time and space. Specifically, these EcoPODs will be equipped with cameras that can, e.g. track plant above-ground biomass over time and rhizotrons that will monitor root architecture and bifurcation patterns under various conditions. Bacterial, archaeal, fungal and phage communities will be surveyed over the course of the experiment with respect to their diversity and activity via DNA and RNA sequencing as well as proteomics and metabolomics analyses. Sampling strategies will be designed to accommodate both temporal and spatial sampling of a number of ecosystem components and environmental parameters will include thorough characterization of above- and belowground space. Sensors will record important parameters for ecosystem development and biotic interactions, including temperature, moisture, gases, and pH. Currently, we are conducting sensor development and EcoPOD prototyping at Potter Street (Aquatic Park), and plant growth and maintenance in growth chambers at the Joint BioEnergy Institute as well as in greenhouses at UC Berkeley.

Figure 5: Rainout shelter, Texas. Photo: Thomas E. Juenger, UT Austin.

2 Isotope Labeling

A key driver in ecosystem health is the movement of carbon from the atmosphere to plants and through the soil and soil microbiome via plant exudates. Researchers, including Mary Firestone of University of California, Berkeley and Berkeley Lab, have pioneered the use of stable isotopes to pulse carbon in a closed ecosystem and measure how it moves through the various trophic levels. Plant-derived carbon enters the soil adjacent to the roots (rhizosphere) where it is consumed, modified, and transported by microbes. Understanding the fate of this carbon is important, as the carbon stock that is present in the soil is orders of magnitude greater than the combined concentration of all the carbon that is present in the atmosphere and land plants. Plants may be labeled with atmospheric $^{13}CO_2$ in an enclosed environment, allowing the isotopically labeled root exudates to be followed in the rhizosphere soil. Microbes that have consumed the stable isotope, either directly from the root exudates or indirectly through the consumption of the primary consumers, can be identified by density gradient centrifugation to separate the heavy "^{13}C" DNA (RNA) from the light "^{12}C", followed by next generation sequencing approaches. Alternatively, a nanoscale secondary ion mass spectrometer (NanoSIMS) can be used to visualize the location of the labeled microbes as well as methods to characterize the labeled functional groups of the metabolites, such as nuclear magnetic resonance spectroscopy (NMR), Fourier-transform infrared spectroscopy (FT-IR), or Raman spectroscopy. The controlled mesocosm environment of the EcoPODs will play a fundamental role in future stable isotope studies of root carbon and the mechanisms of sequestration for long-term soil carbon storage.

3 Multi-trophic Interactions

Ecosystems do not consist of just plants and microbes – fauna are critical for C flow too. Researchers at Berkeley Lab, led by Javier Ceja Navarro of EESA, have been investigating how precipitation affects the composition, function, and interactions among biological compartments (microbial and eukaryotic) of the soil food web and the consequences for C flow in the rhizosphere. For example, as shown in Figure 6, a field experiment was set up for the labeling of Avena fatua plants with $^{13}CO_2$ to then follow the movement of the translocated ^{13}C through the soil food web. Soil microbial and fauna populations are separated (using novel methods developed in the Navarro lab) that are then analyzed with high-throughput sequencing and stable isotope probing techniques.

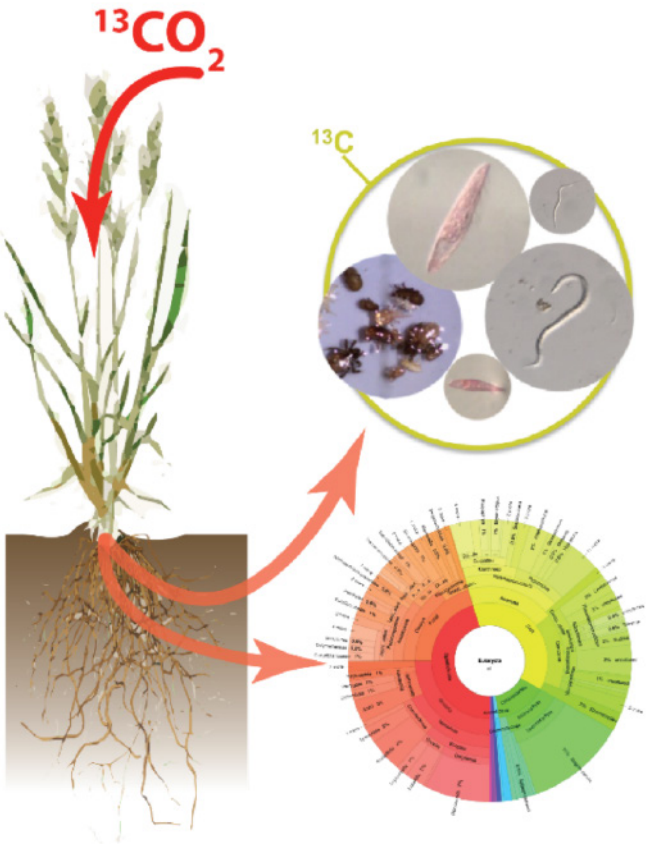


Figure 6: Experiment to feed $^{13}CO_2$ to trace the movement of ^{13}C from plants into microbes and fauna. Image: Javier Ceja Navarro

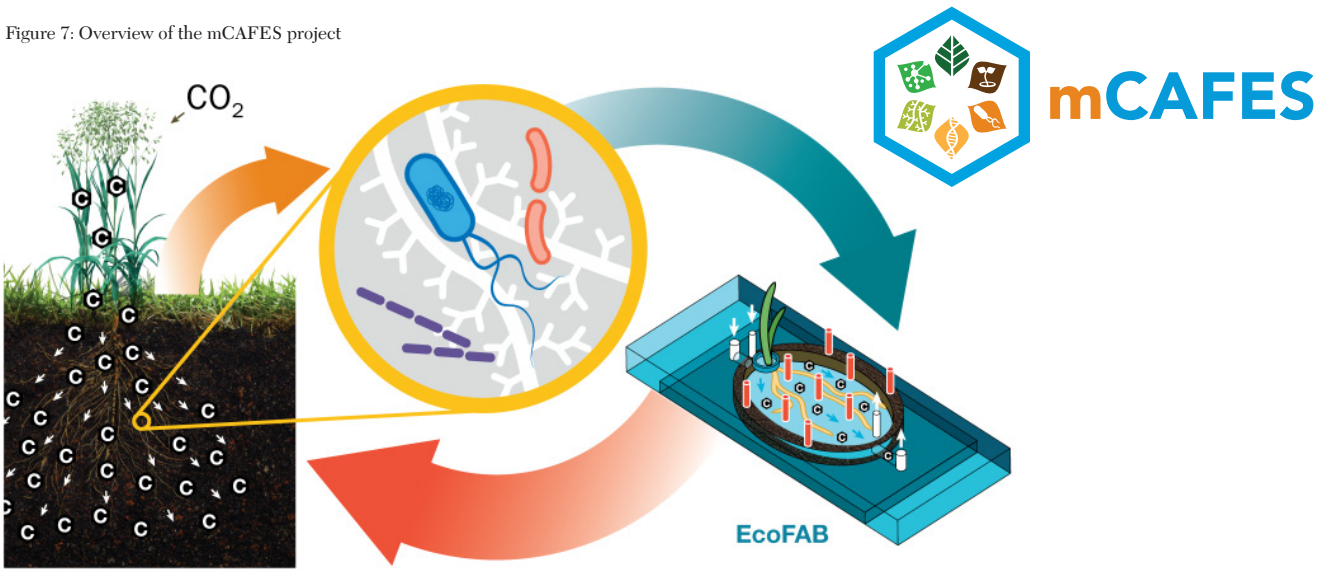
4 mCAFES

The mCAFES project (Figure 7) is a collaborative, coordinated and integrated, mission-driven program to interrogate the function of rhizosphere/soil microbiomes with critical implications for carbon cycling and sequestration, nutrient availability, and plant productivity in natural and managed ecosystems. This will primarily make use and further develop the 3D-printed EcoFABs. An important aspect of EcoFABs is their reproducible control and ability to sample RNA/DNA, metabolites, microbes and to perform microscopy to assess plant root morphological alterations and location of fluorescently-labeled microbes in an ecoFAB. In concert with development of EcoFABs, we are pioneering the development microbial community editing techniques on bacteria and fungi, using CRISPR-Cas and RNA interference (RNAi) respectively. Together, this powerful new platform will provide the fundamental genetic basis for the formation and functional importance of microbial metabolic interaction networks in the rhizosphere/soil. The development of EcoPODs that can house more complex plant communities and soil monoliths is an essential component of the development of ecosystem manipulatories that span the scale from the laboratory, EcoFABs, EcoPODs to field sites. The ability to rapidly perform functional experiments in EcoFABs, which can then be more fully explored in a more complex, but fully controllable, manipulatable, and measurable systems like the EcoPODs is essential for interpretation, testing, and further implementation of field site experiments. The development of enabling technologies across scales to explore microbiome, plant, and ecosystem function directly addresses the DOE Grand challenges of "Understand the biological complexity of plant and microbial metabolism and interfaces across scales spanning molecules to ecosystems" and the development of technologies that "identify DOE mission–relevant metabolic capabilities and engineering possibilities in bacteria, fungi, archaea, viruses, plants, and mixed communities.

5 Finding Engineering Linked Indicators (FELIX)

The IARPA Finding Engineering Linked Indicators (FELIX) program (**https://www.iarpa.gov/index.php/research-programs/felix**) is tasked with developing new methods for detecting engineered biological systems. The EcoPODs provide an opportunity to generate more "real-world" environmental samples for this and other programs with a focus on biosecurity. For example, an engineered microbe can be inoculated into soil containing a native microbiome, plants, and even macrofauna. Samples can be collected over an extensive time period (weeks-months) and can be subsequently interrogated using new biological and computational methods for detection. Similar programs would use EcoPODs to test engineered plants and/or microbes prior to proceeding to field testing, with the associated expense and regulations. Examples at LBNL include plants engineered for improved production of biofuels (Aznar et al., 2018) (as part of the Joint BioEnergy Institute (JBEI) program, **www.jbei.org**) or biosensors which use engineered microorganisms (Zhou, Baruch, Ajo-Franklin, & Maharbiz, 2017).

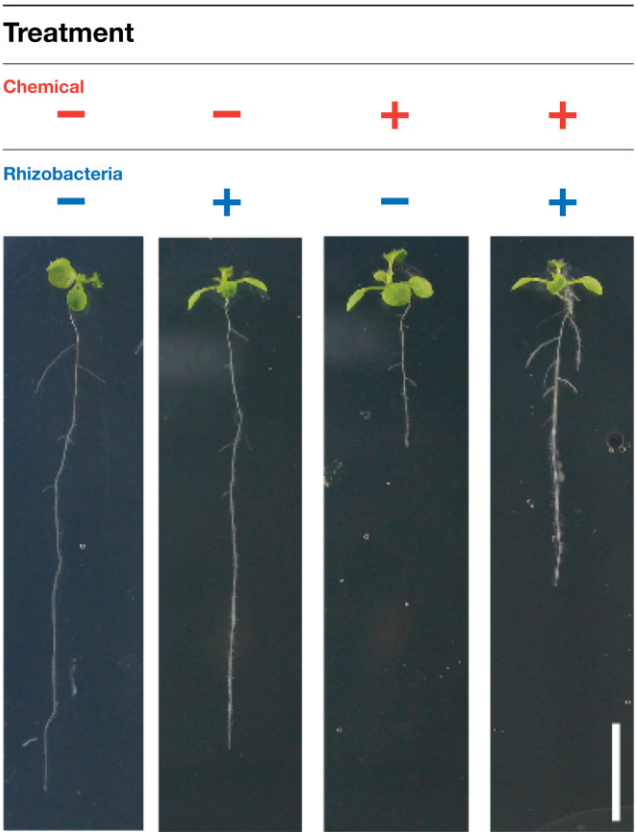
Figure 7: Overview of the mCAFES project



6 Identifying Bioactive Compounds

A team at Berkeley Lab, led by Dr. Mina Bissell has been working to identify bioactive compounds across the tree of life. Making use of specialist assays developed here, combined with computational modeling, our goal is to be able to rapidly identify new activities or toxicities. We have focused on compounds that are of interest in biomanufacturing, where the development is undertaken based on a narrow set of functions related to one application (e.g. a bio-jetfuel), but could have unknown activity for alternative needs (e.g. soil amendments) or have a negative environmental impact (e.g. disruption of plant-microbe interactions). Within this program, the labs of Jenny Mortimer and Aindrila Mukhopadhyay have been exploring the impact of compounds on plants in isolation, as well as in the presence of their microbiomes (Figure 8). The EcoPODs offer the opportunity to test ecotoxicity or bioactivity in more complex ecosystems, without risking environmental exposure.

Figure 8: Effects of a biobased chemical on Arabidopsis root growth, in the presence or absence of rhizobacteria. Image: Robin Herbert



Breakout Session Summaries

For details of charge questions, as well as discussion leads and reporters, see Appendix 2.

Breakout Session I — Science in the EcoPods: What can we do?

1. Data and modeling needs for developing a predictive understanding 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. The ability to manipulate both the above- and belowground environments, and a variety of sensors will lead to terabytes or more of stored data. This aspect will be especially true in long-term experiments in which instrumentation is run at frequent intervals. For long-term efficiency of accessing experimental data, the formats, methods, and results should be standardized across experiments, similar to standardization now being developed for microbiome science in KBase. Substantial computational infrastructure will be necessary for storage and analysis of this data, such as National Energy Research Scientific Computing Center (NERSC). In addition, there will be a need for local computational control within the vicinity of the EcoPOD units that will be used for handling time-sensitive data.

Oftentimes, processes that are observed in the laboratory or greenhouse fail to be reproduced in the field. 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. An interesting aspect of the interrogation of EcoPOD systems is the capability of developing a “digital twin” of a functional ecological model.

2. Defining lab-field gaps of knowledge (microbes, soil, plants) in model systems for carbon cycling and bioenergy.

In situ manipulation of complex soils systems is challenging. There is a need for an experimental set up that can answer wide ranging scientific questions, accommodate the use of current and next generation sensors and create a much needed link between the EcoFAB platform scale and field scale experimentation. EcoPODs provide a unique opportunity to bridge lab-scale observations to *in situ* measurements, and to perform experiments to address soil carbon cycle and plant productivity. EcoPODs can facilitate understanding of effects that cannot be easily untangled in the field such as responses to variations in temperature, moisture, and light that typically covary in nature.

Several plant species were proposed for future experimentation based on their importance to DOE including but not limited to sorghum, Setaria (C4), *Brachypodium distachyon* (C3), *P. hallii*, *Spirodela polyrhiza* (duckweed), *Camelina sativa*, legumes (e.g. clover), and pennycress. Selection of plant species will depend on number of plants that can be housed in each EcoPOD chamber, while avoiding edge effects. Determination of root architecture is important to discretize root effects and to study plant-microbe associations. Microbes of interest include arbuscular mycorrhizal fungi (AMF), *Pseudomonas fluorescens*, and synthetic or native bacterial communities. EcoPODs can be used with artificial soils, monoliths or re-created depth profiles made from natural soils. Consideration of different sterilization methods is of importance, both for creation of sterile soils to study, and soil disposal post-experiment.

Participants discussed potential experiments to complement existing and proposed experiments in the EcoFAB project. This would establish the effects of scale (time and soil volume) between the two systems, but also benchmark the EcoPODs against a very defined field system. Soil horizons from Kearny Field station (UC ANR) will be used to pack EcoPODs where two well-defined, contrasting plant lines will be planted. Kearny soils are an excellent candidate since the soil horizons have been extensively studied, and metagenome and plant data are available, providing comparisons with *in-situ* conditions.

EcoFAB experiments can be integrated into reiterative experiments with EcoPODs via use of natural soil microbial communities in these two systems. Engineered sorghum, such as the drought tolerant lines being developed at JBEI could be tested. Data collection will include plant biomass and phenotypes, microbial community structure, and soil properties and chemistry.

Future research areas:

- Transfer of N from biocrust to plants in arid ecosystems
- Atmospheric VOCs impact on plants and microbes
- Climate adaptation for plants and soil microbes
- Management practices in agricultural systems

Break-out Session II — Design of EcoPODs: What are the basic requirements?

1. Sensors, imaging and environmental controls including data capture

Growing plants under realistic conditions in specially designed enclosed chambers creates challenges but also offer several advantages. To a large extent it is possible to replicate actual environmental conditions, including air, light, hydrology, soil conditions as well as daily and seasonal variations with the right set of instrumentation. To maintain accurate environmental control, the EcoPOD chambers will need to be outfitted with controls for temperature, humidity, light (intensity and diurnal cycle), atmospheric gas composition (air, CO_2), condensation management, and power backups. Placement in a climate-controlled room will simplify the control of boundary conditions.

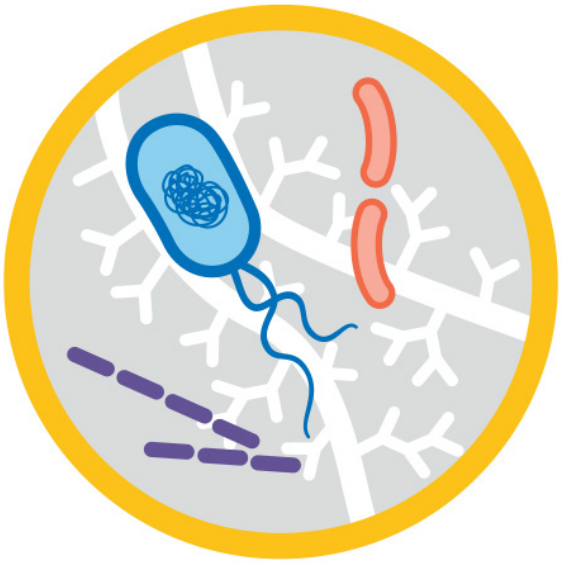
The most appropriate sensors for EcoPODs will be ones that are able to capture key variables required for a predictive understanding and modeling of plant growth under different environmental regimes. A first step in this direction is an analysis of the type of sensors that exist with regards to their resolution, sampling rates, range, and calibration. The prospect of using biosensors as bioreporters is bright. Biosensors that combine biological signals to electrical/digital readouts (Caroline Ajo Franklin, in preparation) will be areas for further collaboration and development for EcoPODs. For atmospheric measurements, canopy level headspace

measurements include in-line MS analysis for VOCs from leaf and other gas phase measurements (CO_2, O_2). PAR sensors for measuring leaf-gas exchange and vertical structure of radiation across canopy can be used, depending on parameters of an EcoPOD experiment. Top mounted cameras can capture visuals of overall plant growth in addition to hyperspectral imaging to determine real-time nutrient status in plant leaves. Photosensors to measure albedo can be held in position with clamps to monitor light transmission vs. reflectance. Measuring sap flow (phloem and xylem) would be very valuable in addition to standard leaf area index measurements.

For the root zone or other belowground measurements, the choice of sensors will depend on the plant (and root system) under investigation. Further developments in biosensors may provide the ability to measure certain metabolites/nutrients in the rhizosphere. New methods to measure 3D root architecture and root density need to be developed. Access ports and windows in the EcoPOD chambers at the rhizosphere level will enable placement of sensors and visualizing belowground root and soil morphology. Key soil measurements include redox vertical profile, imaging of soil structure and soil moisture, pH, and moisture/water potential. Multi-analyte sensors that provide measurements of key soil parameters, such as conductivity, N, O_3, NH_3, and O_2 concentration are highly desired. The leachate from soil of EcoPODs can be captured, enabling measurements of metabolites. In-line spectroscopy analyses for continual measurements should be explored.

2. Sampling strategies for real-time capturing of DNA, RNA and metabolites

EcoPODs will enable temporal and spatial sampling of ecosystem components including multi-faceted datasets of plants, fauna, microorganisms, fungi, phage, soil, and headspace. The biotic components will provide information in the form of abundance/biomass, DNA, RNA, protein, and metabolites that can be used to enumerate, identify, classify, and track growth and diversity over time and space. Bacterial, archaeal, fungal, and phage communities will be surveyed over the course of the experiment with respect to their diversity and activity via DNA and RNA sequencing, as well as proteomics and metabolomics analyses. Various EcoPOD components require measurements and sampling over different time and spatial scales as well as a mixture of destructive vs. non-destructive sampling. Hence an experimental set-up that allows maximum data generation while causing least disruption to the ecosystem possible will be key to EcoPOD experiments. For this reason, we aim to implement sampling techniques and schedules that provide valuable and comprehensive datasets allowing statistical analysis and ensuring insightful outcomes for exploratory as well as hypothesis-driven experiments. This translates, for example, into smallest possible sample volumes to be as least destructive as possible and continuous sampling of non-destructive metrics, such as soil and headspace T, pH, gases, moisture and conductivity.



Summary and Conclusions

Conclusions

Understanding and predicting complex ecosystems is one of the grand scientific challenges of this century, and it will require large scale interdisciplinary collaborations. Linking molecular insights from the lab with field data and models will be critical. The proposed EcoPOD project will enable researchers to work together to develop and test hypotheses about ecosystem interactions and C flow, to make predictions about how ecosystems might respond under altered climatic conditions, and to manipulate different ecosystem components in a controlled, replicated manner. Berkeley Lab's broad range of expertise is uniquely suited to supporting this program of research, as well collaborating with other US scientists from National Labs, academia, and industry.

The EcoPOD workshop assembled a diverse set of researchers, including biologists, engineers, mathematicians, computational scientists, and safety specialists from across Berkeley lab, as well from other National Labs, universities, and NGOs, to consider what an EcoPOD facility would look like, and how it could advance US science. The discussions focused on data collection and ontology, sensor development, as well as the types of ecosystem that could be explored. While soil monoliths should be investigated in the longer term, it was suggested that initial prototyping of the EcoPODs should be done with re-packed soil from the Kearney field site at UC ANR. Grass species, either native or bioenergy-relevant (switchgrass, *P. hallii*, sorghum) were recommended as the first plant species to test in the system, as it would complement existing work at Berkeley Lab (e.g. JGI, JBEI, ARPA-E), and these are well-studied systems in the lab and field. Initial EcoPOD setups should allow as many researchers as possible to explore the system and suggest improvements, technology developments or add-ons.

Sensor development will be critical to the success of this project, as will data collection and management. While extensive and fruitful discussions were had during the workshop, there was a consensus that these topics in particular require further consideration. To support this, we have now established an EcoPOD website (**https://sites.google.com/lbl.gov/ecopods/**) where we are soliciting more feedback. In addition, a sensor development working group, as well as a data and modeling working group will be formed at Berkeley Lab, who will work closely with the EcoPOD development team. We also noted that it was important that the EcoPODs are not developed in isolation at Berkeley Lab. Therefore, an EcoPOD Advisory Group will be formed that has representatives from other interested National Labs as well as other key collaborators. Finally, there was a lot of enthusiasm for engaging the wider DOE science community, through additional workshops with a focus on science questions and technology development, enabling the use of EcoPODs to address grand challenges in environmental research.

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Appendices

Appendix 1: Workshop Agenda

June 7th, 2018

8:30 am	**Registration**
9:00 am	**Welcome, Introduction** Louise Glass
9:05 am	**EcoPod Concept** Jenny Mortimer, Gary Andersen
9:25 am	**Talks** *From Lab to Field to Lab, A Perspective from PNNL* (Christer Jansson, PNNL) *EcoFABs — Single Plant Scale Fabricated Ecosystems* (Peter Andeer, JGI, LBNL) *EcoSIM/EcoTECH — Suggestions for EcoPOD Plan-Soil-Microbe Modeling* (Bill Riley, LBNL)
10:05 am	**Pop-up Talks** *EcoSense - Integrity and connectivity across scales* (Yuxin Wu, LBNL) *Soil Multitrophic Interactions Shape Ecosystem Functioning* (Javier Ceja Navarro, LBNL) *Improving biofuel yield from field to lab* (Esther Singer, LDRD awardee, LBNL)
10:30 am	**Break**
10:45 am	**Break-out Session I - Science in the EcoPODs: What can we do?** 1. Data and modeling needs for developing a predictive understanding of dynamic biological processes. 2. Defining lab-field gaps of knowledge (microbes, soil, plants) in model systems for carbon cycling and bioenergy.
11:30 am	**Report out**
11:50 am	**Group Photo**
11:55 am	**Working Lunch** *Using stable isotopes to follow carbon use in the rhizosphere* (Mary Firestone, UCB, LBNL)
1:00 pm	**Break-out Session II - Design of EcoPODs: What are the basic requirements?** 1. Sensors, imaging and environmental controls including data capture. 2. Sampling strategies for real-time capturing of DNA, RNA and metabolites
1:45 pm	**Report out**
2:05 pm	**Break**
2:20 pm	**Discussion, Next Steps, Meeting report**
3:00 pm	**Adjourn**

Appendix 2. Workshop Charge Questions

Data and Modeling Needs

Discussion Leader: Paramvir Dehal; Scribe: David Donofrio

- EcoPODs will generate a large amount of data during experiments – how will this be stored,managed (monitoring of EcoPOD environmental conditions, sensor outputs, images etc, 'omics)?
- How will this data be shared, and made available to the wider community?
- What data should we be collecting?
- Any special aspects of the EcoPOD that should be constrained to improve computational modeling efforts?
- Are there existing modeling efforts at the lab who would be interested in working at this scale with this replication?
- How can we link sensor data so that it feedbacks to environmental control of each unit?

Defining Lab-Field Gaps of Knowledge

Discussion Leader: Susannah Tringe; Scribe: Neslihan Tas

- Which models?

  Plant species, microbial communities (native, synthetic), soil types (monolith, field soil, artificial soil…), other ecosystem additions (e.g. arthropods, fungi?)
- Which questions can we answer at this scale that cannot be addressed adequately in the lab or field?
- Which topics should we target that are of high DoE relevance and fit with existing or upcoming LBL priorities?
- What's needed to follow carbon through the system?
- Can you envision the first "big" experiment – how could we involve multiple teams and answer multiple science questions with one EcoPOD cycle running all chambers in parallel
- What do you need to measure? What data do you absolutely need? What would be nice in addition?
- How can we validate our data e.g. benchmark to field?

Sensors

Discussion Leader: Sergio Zimmerman; Scribe: Romy Chakraborty

- What can we measure? What sensors exist?
- What sensors can you envision being developed or adapted for this system? Could these be used in tandem with engineered microbes, plants (e.g. fluorescent reporters)
- How might we access the soil? Non-destructive, destructive?
- What type of imaging (above, below ground)?
- What environmental controls are necessary? What range should we be looking at e.g. temperature, CO_2?

Sampling Strategies

Discussion Leader: Trent Northen; Scribe: Esther Singer

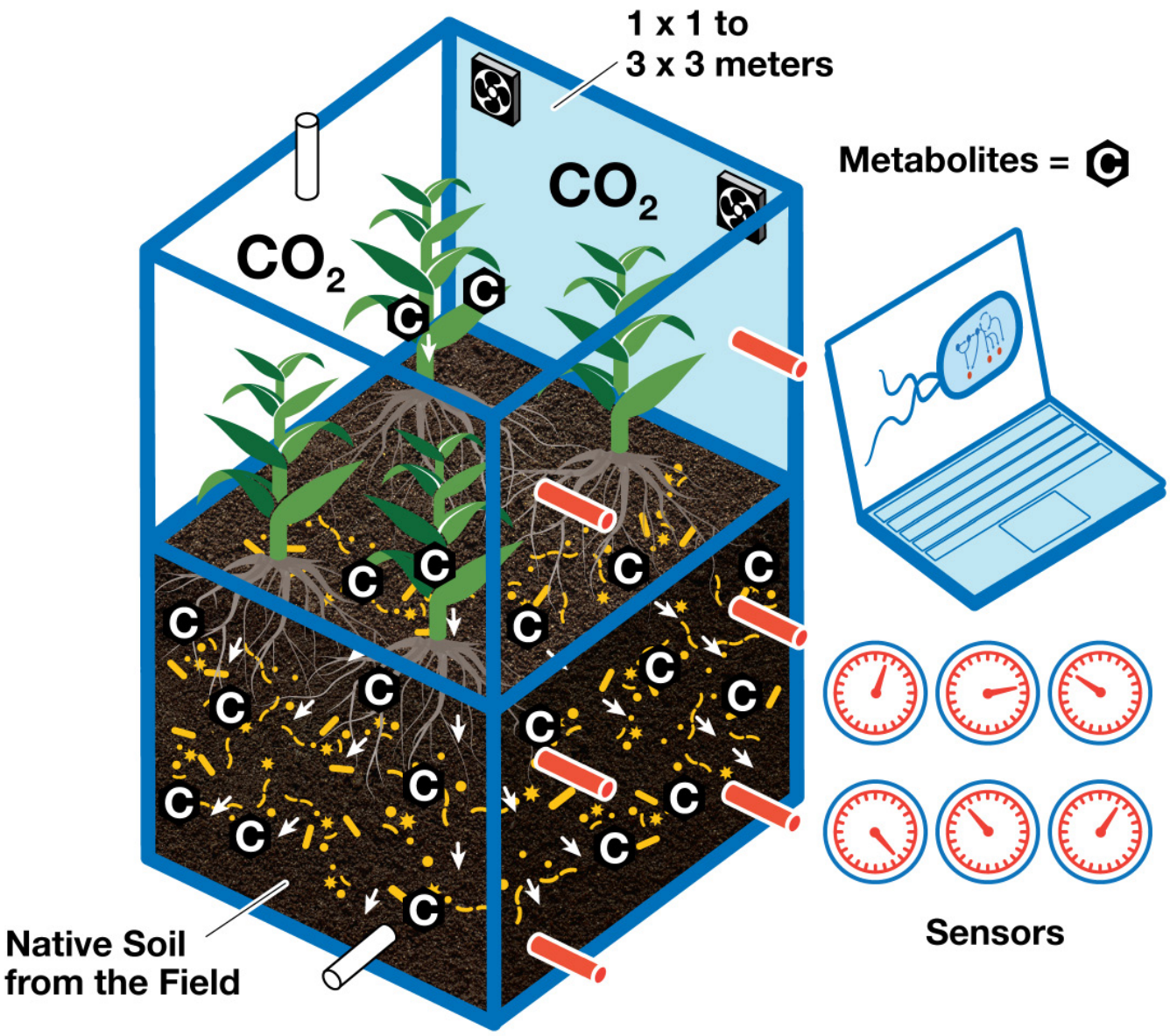
- How might we access the soil?
- How do you know where you're sampling e.g. root adjacent or not?
- What is needed for sampling? Time-sensitivity, sample quantity, sample storage, degradation concerns, inhibitors?
- Replication, statistics – what is the minimum requirements?
- New technologies that might support *in situ* measurements? Reporters?

Appendix 3: Workshop Participants

Workshop Group Photo

Last Name	First Name	Division/Title	Affiliation
Aghajanzadeh	Arian	Energy Technologies	LBNL
Ajo-Franklin	Caroline	Molecular Foundry	LBNL
Ajo-Franklin	Jonathan	Earth & Environmental Sciences	LBNL
Amen	Sheevah	Environmental Genomics & Systems Biology	LBNL
Andeer	Peter	Environmental Genomics & Systems Biology	LBNL
Andersen	Gary	Environmental Genomics & Systems Biology	LBNL
Bouskill	Nick	Climate & Ecosystems	LBNL
Brodie	Eoin	Climate & Ecosystems	LBNL
Brown	Ben	Environmental Genomics & Systems Biology	LBNL
Canon	Shane	NERSC	LBNL
Carey	Chelsea	Sr. Soil Ecologist	Point Blue Conservation Science
Ceja Navarro	Javier	Climate & Ecosystems	LBNL
Chakraborty	Romy	Earth & Environmental Sciences	LBNL
Crivelli	Silvia	Computational Research	LBNL
Dafflon	Baptiste	Climate & Ecosystems	LBNL

Last Name	First Name	Division/Title	Affiliation
Dehal	Paramvir	Environmental Genomics & Systems Biology	LBNL
Denes	Peter	Directorate Office	LBNL
Donofrio	David	Computational Research	LBNL
Duarte	Robert	Engineering	LBNL
Firestone	Mary	Environmental Sciences and Policy Management	UCB
Garcia-Martin	Hector	Biological Systems & Engineering	LBNL
Gentz	Reinhard	Computational Research	LBNL
Glass	Louise	Environmental Genomics & Systems Biology	LBNL
Gunter	Dan	Computational Research	LBNL
Jansson	Christer	Plant Sciences	PNNL
McKenzie	Jenna	EH&S	LBNL
Mortimer	Jenny	Environmental Genomics & Systems Biology	LBNL
Mukhophadhyay	Aindrila	Biological Systems & Engineering	LBNL
Nico	Peter	Energy Geosciences	LBNL
Northen	Trent	Environmental Genomics & Systems Biology	LBNL
Riley	Bill	Climate & Ecosystems	LBNL
Simmons	Blake	Biological Systems & Engineering	LBNL
Singer	Steve	Biological Systems & Engineering	LBNL
Singer	Esther	JGI	LBNL
Stuart	Rhona	Biochemical & Biophysical Systems Group	LLNL
Tas Baas	Neslihan	Climate & Ecosystems	LBNL
Tringe	Susannah	JGI	LBNL
von der Lippe	Henrik	Engineering	LBNL
Wu	Yuxin	Climate & Ecosystems	LBNL
Zimmermann	Sergio	Engineering	LBNL



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