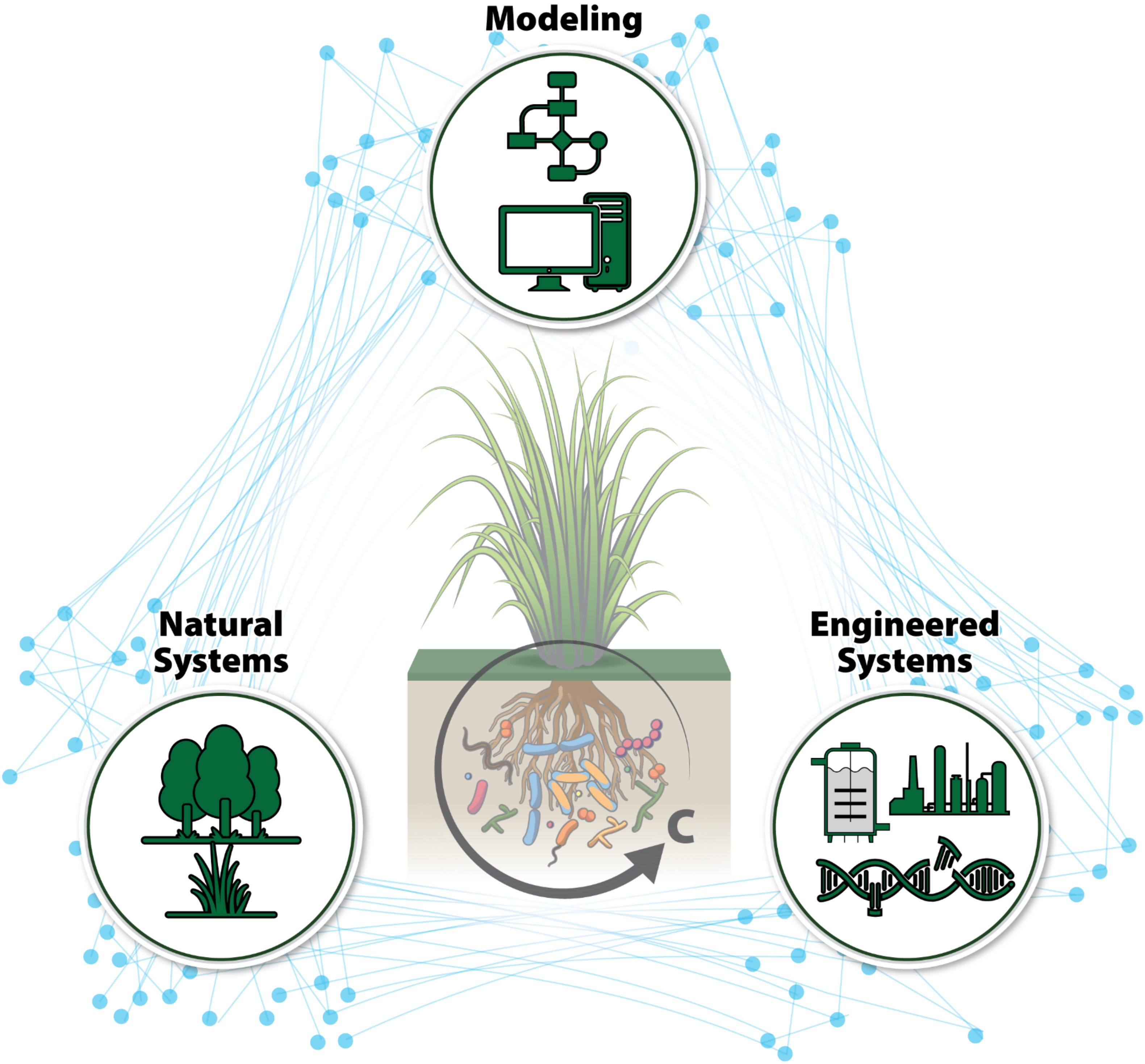


Modeling
Natural
Systems
Engineered
Systems
C

Cover Image: Carbon cycling and biological research is part of a vast network of interactions where all network nodes are needed to achieve the goal of biological carbon sequestration for climate and soil health.

Biological Carbon Sequestration for Climate and Soil Health

Report from the virtual workshop on Biological Carbon Sequestration for Climate and Soil Health
October 19, 2021

Organizing Committee (alphabetical by first name):

Aymerick Eudes, Deputy Director, Cell Wall Biology & Engineering Group, Joint Bioenergy Institute and Scientist, Environmental Genomics and Systems Biology Division, Biosciences Area, LBNL

Erin Webb, Senior R&D Engineer and Group Leader, Bioresource Science & Engineering Group, ORNL

Kateryna Zhalnina, Research Scientist, Environmental Genomics and Systems Biology Division, Biosciences Area, LBNL

Julie Mitchell, Division Director, Biosciences Division, ORNL

Katy Christiansen, Area Science Deputy and Head, Strategic Program Development Group, Biosciences Area, LBNL

Lauren Jabusch, Program Developer, Strategic Program Development Group, Biosciences Area, LBNL

Xiaohan Yang, Senior Scientist, Synthetic Biology Group, Biosciences Division, ORNL

Contents

1. Executive Summary

The United States recently launched the Net Zero World Initiative on decarbonization. As temperatures and sea levels rise, strong action is needed to mitigate high atmospheric CO_2 levels. In response to this need, a joint workshop between the US Department of Energy's Oak Ridge National Laboratory (ORNL) and Lawrence Berkeley National Laboratory (LBNL) was convened to assess the challenges and opportunities within biological carbon (C) capture.

The virtual workshop on Biological Carbon Sequestration for Climate and Soil Health focused on three aspects of biological C capture research: natural systems, engineered systems, and modeling. For this workshop, the scope of natural systems included unmanaged (e.g., grasslands, wetlands, natural forests) and managed (e.g., agricultural lands, commercial forests, forest biofuels) ecosystems. The engineered systems group covered topics in genetically modified plants and microbes, synthetic microbiomes, and altered plant–microbe interactions for enhancing C capture, storage, and utilization. The modeling group focused on computational tools and data collection to predict and assess the effectiveness of new biocarbon sequestration advances. Participant expertise included plant and microbial systems biology, synthetic biology, soil biogeochemistry, and techno-economic analysis. Together, participants outlined key scientific advances in support of biological C capture and storage.

Among the major findings of the meeting were the need to better measure C flux within natural, agricultural, and fabricated ecosystems for experimentation; better understand the role of soil composition, plant roots, and the rhizosphere (i.e., the soil surrounding the plant root) in the underground storage of organic C; and design plant–microbe systems for maximal C storage.

To achieve the goal of C capture through biological means, research opportunities are as follows:

- *Plant and microbial phenotyping*—investments are needed to pinpoint the genomic controls and traits of plants and microbes that affect the storage of soil organic C (SOC). High-throughput methods for aboveground and belowground plant phenotyping along with microbial (community) phenotyping are required.

- *Soil biogeochemistry*—a better understanding of soil health and its relationship to plants and microbes is needed to utilize lands toward biological C capture, and to determine how to engineer long-term storage of C in soils.

- *Fabricated ecosystems*—investments are needed to enable the study of plant–microbe–soil systems within tunable and controllable environments. Fabricated ecosystems (standardized microcosms or mesocosms) facilitate the tracking of C fluxes and quantification of root exudates under prescribed conditions.

- *Synthetic biology and design, build, test, learn (DBTL) cycles*—synthetic biology and DBTL cycles can drive the future of C capture through rapid prototyping and hypothesis testing, such as for deeper plant roots and improved hardiness of cover crops. Artificial intelligence/machine learning (AI/ML) can help bridge existing models among scales, genomes, and traits.

- *Advanced sensor networks and data–model integration*—modeling and controlling C fluxes at the field scale will require new technologies for monitoring gas fluxes and converting measurements into parameters within ecological simulation models.

Through a focused research effort, scientists can enhance the earth's ability to capture and store C underground. Research opportunities at the interface of ecology and synthetic biology can develop the needed toolkits for engineering plant–microbe systems of direct climate value.

2. About the Workshop

On October 19, 2021, ORNL and LBNL jointly hosted a virtual workshop on Biological Carbon Sequestration for Climate and Soil Health. The workshop built on past jointly hosted workshops and collaboratively written white papers. The workshop had three goals:

(1) Team building: understanding the ORNL and LBNL research expertise in biocarbon sequestration, including commitment level and potential for collaborations

(2) Applying an engineering approach to idea generation and proposed science to evaluate the success of C sequestration using DBTL cycles

(3) Information collection on three themes: natural systems, engineered systems, and modeling and economics for biocarbon sequestration.

The 30 participants represented ORNL and LBNL. They brought expertise in microbiology and microbial engineering, plant biology and plant engineering, soils, sensors, and computation and modeling (including techno-economic analysis, life cycle assessment, AI/ML, and biogeochemical models).

The scientific focus of the workshop was set by two science talks. First, Corinne Scown (LBNL) presented on Life Cycle Analysis and Techno-Economic Analysis for Biocarbon Sequestration to guide ideation on DBTL discovery cycles using environmental and economic models. Second, Jerry Tuskan (ORNL) presented research and principles on Plant Science for Biocarbon Sequestration to inspire the biology-based visioning. After the science talks, participants had a discussion on identifying targets, obstacles, and measurements for C sequestration. Participants had a second discussion in breakout groups that focused on natural systems, engineered systems, and modeling and economics.

There has been much debate on the definition of biological natural systems vs. engineered systems in preparation for the workshop, during the workshop, and in writing this report. The information presented here, and the organization of this report, reflects the topics discussed in the small groups under this imperfect division, and we expect the definition of these fields of biology to shift and converge over time.

Some breakout groups formed main ideas, such as what is already known in the field, problems that can only be solved with a team science approach like at the national labs, potential outcomes in 5–10 years, and translating discoveries and technology to real-world application. Subsequently, other breakout groups took a round-robin approach to review and contribute to the first breakout groups' findings. This report describes the findings from all of these discussions. This workshop was funded with internal Strategic Program Development funds at ORNL and LBNL.

3. Introduction and Motivation

The window of opportunity for action on climate change is quickly narrowing. Action is urgently needed to prepare, repair, and control for the most detrimental effects of climate change. An opportunity for action is to sequester C using biological processes into durable, long-lived, biologically derived goods, and to sequester C in the soil. In addition to having climate benefits, biological processes are value-added processes. The transition away from fossil fuels leaves a materials gap that could be fulfilled with biobased products, actively removing C that was once emitted through the production of similar goods. Furthermore, sequestering C in the soil improves soil health and fertility.

Three potential targets for biologically driven C sequestration were identified in preparation for this workshop: natural systems, engineered systems, and modeling. In natural systems, C can be effectively stored in plant biomass and soil. Soil C storage potential is estimated to be large but is difficult to quantify because of technological constraints. Soil can sequester C longer than in standing plant biomass, and some forms of C have longer residence time. Of course, soil and plants are not separate systems, and plant–microbe and microbe–microbe interactions mediate C flow and stabilization.

There are some well-established microbial systems for CO_2 conversion and C1-based biomanufacturing. Designed microbial systems, including engineered microbes and purpose-built microbial cocktails, for C capture are emerging and have important potential. Plants have been engineered effectively for rapid growth, pest resilience, and yield, but limited research has been conducted on engineering plant biochemistry to promote C sequestration. Modeling is an important part of the DBTL cycle to place knowledge in rigorous frameworks and can accelerate and provide crucial feedback to researchers in both natural systems biology and biological engineering. Modeling methods such as detailed techno-economic analysis for engineering design and construction are well established (ASPEN [Advanced System for Process Engineering] is a well-known example). Life cycle assessment and similar methods can model biofuels and bioproducts production, as well as agricultural emissions associated with farm inputs and operations. Emerging methods in AI/ML models are needed for accurate predictions, and they show promise for improving mechanistic models that are not yet accurate for biological processes. On the macroscopic scale, large-scale earth system models for climate are continually improving, but these models have gaps in biological model components, especially microbiological components.

Over the course of the workshop, participants considered where the national labs have the biggest opportunities to improve natural systems biology, biological engineering, and modeling. National labs play a central role in the C sequestration research landscape. In particular, national labs have the ability to coordinate and execute large, targeted, interdisciplinary, and team-based research that is otherwise unattainable at most dispersed research institutions. The following workshop insights considered the well-established science and capabilities of the national labs when examining the potential for impactful C sequestration science.

4. Workshop Insights

Carbon sequestration: targets, obstacles, and measurement

Participants identified C storage targets in five major groups: soil and microbes, belowground plant biomass, aboveground plant biomass, durable bioproducts, and other targets. For soil and microbes, the C storage involved dead organic tissues and plant detritus (e.g., lignin and similar compounds, soil humus), mineral-associated C (e.g., metabolites, proteins, extracellular polymeric substances), and mineralized C. Additionally, conditions for stabilizing C, such as environments to restrict microbial activity (e.g., pH, low water, low oxygen), were identified. Methods of microbial community engineering, such as enzyme engineering for diverse, low-abundance metabolites that would be difficult to degrade, should be considered as targets for research, but more research is needed to confirm that these chemicals sequester C in the soil. Another approach is to accelerate C flow by increasing microbial biodiversity and microbial trophic associations. Carbon storage concepts in belowground plant biomass included deep roots, recalcitrant C forms such as aromatics and suberins, and plant engineering for increasing microbial symbiosis. Aboveground plant biomass could include standing forest stocks, and strategies included enhancing photosynthesis, improving plant water usage, and increasing plant resilience to grow in less productive lands to expand productive regions. Durable bioproducts identified were building materials, plastics, wood, and fungal-mycelia products. Finally, ideas that fell outside these categories were aromatic C, harvesting CO_2 from biorefineries, and biochar.

Overall, the most significant concerns for many of the storage targets included the ability to accurately measure C residence times in soil, and to predict residence times in complex environments. For soil and microbial systems, other obstacles included the complexity of the soil, the lack of top-down controls in soil studies, and stability of C in soil under different environmental pressures, such as near the soil surface and under climate change effects. Belowground plant biomass obstacles included high-throughput phenotyping systems, understanding the fate of plant-derived C in the soil, trade-offs with yield, and soil storage capacity for plant-derived exudates. For aboveground plant biomass, the major obstacles included fire risk, changing patterns of environmental disruption, duration of sequestration, and amount of land available (including food vs. fuel concerns). Durable bioproducts have obstacles in the manufacturing scale, meeting product performance requirements, and maintaining durability (i.e., microbial evolution to consume "durable" products). Other obstacles focused mostly on policy and public perception, such as land use change, stable C markets and incentive systems, the public's understanding of biomass harvest and use, and green premiums (i.e., the difference in the upfront cost of using a clean technology vs. a technology that produces greenhouse gas emissions, with potential long-term ramifications).

The workshop participants extensively discussed the challenge of measuring dynamic C flux. Chemical strategies included the use of ^{13}C-labeled plants and stable isotope analysis of metabolites, and improved methods in SOC analysis and metabolomics. A suggestion was made to conduct large-scale genome-wide association studies and/or field trials to destructively harvest for belowground traits There were also calls to improve and expand nondestructive sampling methods with sensors, such as new imaging methods with long penetration depths that can be deployed in the field to view belowground dynamics. Sensing included microbial methods such as microbial sentinel biosensors and microbial community monitoring. Chemical sensing comprised real-time sensors for greenhouse gasses and soil C. Participants also discussed pairing agricultural equipment with sensors such as soil C sensing on tillage equipment and adapting precision agriculture for dynamic sensing. More advanced technologies, including robotic samplers such as ground or aerial drones and satellite monitoring of photosynthesis, were also discussed. The data generated from these experiments and sensors would feed into predictive models, such as calibrated explainable AI (XAI) models. Improvements that are still needed in both simulation and AI models include uncertainty calibration, model validation, and geospatial mapping tools. Overall, the participants viewed this discussion as an opportunity to experiment, track, and model C flow through substrates.

In the last discussion before splitting into breakout groups, participants considered how to integrate DBTL cycles into methods to manage dynamic C flux. Researchers need to have a basic understanding of the role of fine root turnover (as a source of C and as an ecological niche for plant–microbe interactions). In this designing stage, a deep comprehension of the important chemical and microbial players to increase C longevity in soils is needed. In the building phase, sensors—particularly those that are paired with existing precision agriculture infrastructure or that could be produced at low cost—would need to be deployed alongside plants engineered with reduced degradation or root exudate profiles and the plant's corresponding microbial amendment. These sensors would feed into the next generation of C management models, including XAI models to determine drivers for C stability in soil. In the learning phase, the resulting models could identify regions amenable to deep C sequestration, and arid regions are of a particular scientific and environmental interest. Through successful iteration(s) in the DBTL cycle, this research could result in important policy and economic findings, including land use planning and valuation of C for agriculture and land managers.

Breakout Group 1: Natural Systems

Overview

Natural systems include unmanaged (e.g., grasslands, wetlands, natural forests) and managed (e.g., agricultural lands, commercial forests, forest biofuels) ecosystems where organisms and abiotic components interact with one another and their environment and are shaped by climate, geography, and human activity. Ecosystems exist along a continuum of management, from unmanaged to moderately managed (such as in forestry) to highly managed (such as agricultural fields). In this workshop, *natural systems* were defined to include ecology and agrosystems and exclude synthetic biology (see engineered systems in Breakout Group 2). Climate change—including elevated temperatures, increased frequency of droughts, and increased risk of wildfires—significantly impacts natural systems' properties and alters vital ecosystem services, such as C sequestration. Natural systems can provide efficient solutions to mitigate climate change by removing CO_2 from the atmosphere and storing it belowground. In addition to the challenge of improving C sequestration and stabilization, land use change to satisfy growing food and energy demands may negatively impact C storage. Therefore, the full potential of natural systems must be used to store C belowground, and consequently, a better understanding of how to increase the long-term stabilization of soil C in natural systems is needed. The interactions among plants, microorganisms, and environmental factors (e.g., soil chemistry, physical structure, porosity, minerals) are the central contributors that influence SOC dynamics and affect C stocks. However, the specific mechanisms of C stabilization in soil, particularly over the long term, are difficult to predict because of the high complexity of natural systems.

To overcome this challenge, new technologies are needed to measure C transformation processes and analyze associated C pools, in managed and relatively undisturbed natural settings, to account for all variables existing in soils. Surprisingly little is known about the precise chemical composition of soil C pools and the relationship between the structure of organic molecules and their residence time in soil. Integration of stable isotope approaches with metabolomics has the potential to address this knowledge gap. In addition, most approaches do not account for spatiotemporal heterogeneity of C transformation processes. Studying C cycling from the soil pore size scale to the ecosystem-level scale, including C transformations below 1 m depth, will improve our knowledge of spatial distributions of C stocks and factors affecting C storage at these scales. New capabilities are needed to bridge different scales and parameterize ecological models. This need requires field-to-lab studies in which new technologies, including machine learning and instruments that simulate field experiments in the lab, will allow measurement and prediction of environmental processes such as C fluxes under more realistic but highly controlled conditions.

Diversity of ecosystems, including peatlands, grasslands, savannahs, forests, and agricultural lands, must be considered to understand how the complexity of C cycle results in different C stocks across natural systems in the United States. Addressing these challenges requires leveraging multidisciplinary teams from soil science, biogeochemistry, microbial and plant genetics, and ecology; establishing unmanaged and managed systems as model systems to study the processes regulating the C cycle, including long-term experiments allowing access to long-term C storage; systematically assessing C sequestration strategies across many environments; iterating among laboratory and natural systems; and implementing high-performance computing/modeling to build a holistic perspective for complex processes defining the fate of soil C. State-of-the-art technologies are needed to address these challenges, and some of these technologies are being developed by LBNL and ORNL teams. These include

- Fabricated ecosystems (including rhizosphere-on-a-chip, rhizoboxes, soil microcosms, EcoFABs, EcoPODs, and the EcoBOT) that bridge the gaps between lab- and field-scale experiments and allow for measuring C fluxes, and imaging the spatial distribution of roots and root symbionts, collecting samples to measure soil C chemistry and microbial dynamics across space and time under field-relevant conditions;

- Approaches to measure and characterize composition of SOC, including spatial distribution of C in soil and rhizosphere;
- High-throughput phenotyping for plants and microbes, such as the Advanced Plant Phenotyping Laboratory; and
- Optimized proteomic, genomic, metabolomic and informatic methods to predict microbial function in the field and in isolation.

Technological advancements that are underway and multi–national lab team efforts have a strong potential to advance our understanding of the integrated role of the plant–microbe–soil continuum in altering SOC trajectory in natural systems and increasing the predictive power of ecological models. These efforts will facilitate the development of deployable plant–microbe combinations that enhance CO_2 sequestration, more sustainable soil management practices, and managed land use through the addition of environment-specific plants, microbes, and chemicals, leading to increased C stocks.

Soils

Soils have a tremendous storage capacity, and additionally, many soils have lost C because of land use change, erosion, and other anthropogenic modifications. Consequently, there is a strong potential for soils to be able to take up additional C. However, agricultural management has altered the formation of new SOC. Specific plant and microbial traits can likely be exploited to further increase SOC, particularly in bioenergy and agricultural cropping systems. However, a major constraint is the limited understanding of controls over the chemical forms and residence times of SOC. For example, soil aggregation is a physical process by which soil minerals and particulate organic matter become closely associated into hierarchical structures involving macroaggregates and enclosed microaggregates. The overall effects of plant tissue stabilization as particulate organic matter within soil structures—including the role of tissue chemistry, the extent of shoot vs. root inputs, soil texture, and soil depth, as well as climatic variables such as precipitation and temperature—are currently poorly understood. Dissolved organic C from plant root exudates, microbial decomposition processes, and microbial necromass can become adsorbed onto soil minerals, where it may be stabilized for decades to centuries in the form of mineral-associated organic matter. However, the overall effects of the microbial community are not well characterized, especially under natural and changing conditions. Carbon at depths of more than a few meters remains sequestered on geological time scales, but the rate at which dissolved organic C sinks under hydrological forcings in various soil types is almost completely unknown and necessitates further interrogation.

Microbes

Microorganisms play central roles in processing soil C and promoting C capture by plant communities through enhancing nutrient availability and soil fertility. However, systems-level understandings of soil microbiome function, which are a prerequisite for efforts to enhance crop productivity and SOC accumulation and residence time for sequestration goals, are limited, partly because of the vast diversity of soil microbiomes. Therefore, a central goal of future research must be to advance the tools of microbiome science and systems biology that have emerged in the past decade to understand the microbial roles in soil C sequestration and identify the individual microbial species and characteristics of the microbial communities associated with patterns of robust SOC accumulation across diverse ecosystems. Such efforts will require coordination across research projects and databases such that both diverse ecosystems and microbes are represented, and that important metadata for improving our understanding of soil processes are captured. Because of the intrinsic diversity of soil microbiomes, understanding how to effectively manipulate them for greater sequestration of SOC is also challenging. Strategies have been proposed for direct manipulation through the addition of identified beneficial organisms and communities (e.g., bioaugmentation). However, the effective establishment and persistence of target organisms remains challenging because of the diversity of organisms, ecosystems, and environments. Therefore, bioaugmentation will likely require a large degree of customization. Fabricated ecosystems provide a powerful new lab capability to experimentally test biological and environmental controls on soil C pools and are compatible with metabolomic, genetic, and sequencing technologies to understand the microbial processes that affect soil C pools. Another promising strategy worthy of further investment is the indirect manipulation of natural microbiomes through manipulation of C inputs and plant traits from crops to promote the growth of functional classes of organisms with similar traits desired for SOC accumulation, which will require cross-disciplinary systems-level studies and understanding. Finally, although microbially explicit simulation models for understanding SOC patterns in various ecosystems have developed rapidly in recent years, these models still lack many important components of soil systems and validation against experimental data. In particular, efforts are needed to increase our conceptual understanding and modeling frameworks for incorporating the critical roles played by fungal communities, both as key SOC degraders and as symbionts promoting plant C accumulation.

Plants

Ultimately, plants are the drivers of soil C dynamics because of their role in fixing C from the atmosphere through the process of photosynthesis. The amount of C fixed is commonly referred to as *primary productivity*, and maximizing this productivity provides the C fodder needed for soil sequestration through feeding the microbial community as described below in the Symbiosis section. In many ecosystems, biodiversity of functional types such as herbaceous, forbs, legumes, and woody plants likely enhances productivity through reduced competition and niche complementarity. Photosynthesis itself operates inefficiently, so gains in productivity could be made using breeding or genetic modification to enhance photosynthesis while simultaneously decreasing C use of the plant in potentially wasteful processes, such as root respiration. Most soil C likely derives from root C, so further understanding of shoot–root biomass allocation is important. Tissue composition and abundance are important regulators of microbial processing. Selecting for relatively recalcitrant (or difficult-to-decompose) tissue with compounds such as lignin or suberin to increase soil C may seem promising, but many compounds can eventually be broken down, and most soil C is derived from microbial necromass. Therefore, simply maximizing inputs of recalcitrant tissue as the primary strategy requires further consideration in the context of microbial C use efficiency and other processes such as rhizosphere priming. Rhizosphere priming is a potential risk by which fresh C inputs promote microbial respiration that actually reduces the pool of previously stored C. Root exudates are another important C input that are typically quickly used by soil microbes. Evidently, the situation is nuanced and deserves careful study.

Cross-disciplinary teams are needed to study these processes across scales to understand which traits should be engineered into plant systems. In managed systems, agronomic practices such as reduced tillage and nitrogen management that reduced release of NO_x compounds need to be adopted simultaneously. Ultimately, the context and nuances of controlling soil C storage will need to be explored in detail before recommendations can be made.

Symbiosis (plant–microbe interaction)

Plants release large quantities of photosynthetically derived C into the rhizosphere. This exuded C consists of both simple C-containing molecules and more complex secondary metabolites. Plant exudates shape the rhizosphere microbiome and mediate activity of microbes living around the root, thus affecting biogeochemical cycles controlled by these microbes. Some exudates may recruit specific microbial species or inhibit the growth of others, allowing the plant to partially determine the microbial community structure. Exudates metabolized by microorganisms will enhance overall microbial activity, increasing the production of microbial necromass and microbially derived metabolites that contribute to the soil mineral–associated organic C pool. Alternatively, exudates could stimulate microbial decomposition of SOC and lead to the destabilization of C stocks (i.e., rhizosphere priming). Stoichiometry of key nutrients in soil could significantly affect the extent of rhizosphere priming for key nutrients and thereby either promote SOC stabilization or facilitate SOC loss. The connections among C stabilization/destabilization processes and specific plant exudates, microbial taxa, trophic interactions, associated C cycling microbial genes, and nutrient stoichiometry have yet to be extensively explored.

Specificity and functional diversity of plants and their symbiotic microorganisms and fungi and how they affect C cycling in soil are not well understood. Another major challenge includes advancing our knowledge in the roles and trade-offs of different types of mycorrhizal fungi in soil C cycling by contributing to and mining SOC, particularly under suboptimal conditions. Finally, to gain a holistic view on key biological drivers of C cycling, a full breadth of microbial diversity representing cross-kingdom multitrophic interactions with plants should be considered, including archaea, bacteria, fungi, and protists.

Breakout Group 2: Engineered Systems

Overview

In this workshop, *engineered systems* were defined as genetically modified plants and microbes, synthetic microbiomes, and altered plant–microbe interactions for enhancing C capture, storage, and utilization. Current natural systems cannot meet the requirements of climate change mitigation because of their limited capacity for photosynthetic capture of atmospheric CO_2 and long-term storage of captured C in the soil. Currently, there is an annual net airborne emission of 17 Gt CO_2, and the annual land C sink is 12 Gt CO_2. Recent advances in biotechnology, particularly synthetic biology and genome editing, bring new opportunities for bioengineering to enhance C sequestration mediated by plants and associated microbes, with a goal to increase the capacity of biocarbon sequestration by at least 100% (i.e., annual increase of 12 Gt CO_2 storage through terrestrial systems) to cover a significant portion of the deficit (17 Gt CO_2) in global C availability. The scope of engineering biosystems for C sequestration can be focused on four aspects:

- Plant engineering for enhancing CO_2 capture and utilization
- Microbial engineering for enhancing long-term C storage
- Engineering of the symbiosis between plants and microbes for increasing both C capture and storage
- Engineering of the symbiosis between plants and microbes for increasing dissolved organic C to increase the rate at which C sinks into the deep subsurface

Large-scale deployment of genetically enhanced biosystems with increased capacity of C sequestration is critical for achieving negative C emission and boosting a C-neutral bioeconomy. For this purpose, engineered microbes and plant C sequestration mechanisms that work across scales and geographic regions must be designed. Biosystems design in plants and microbes across scales can be facilitated by high-throughput phenotyping, big data, and exascale advanced computing. However, deployment of engineered organisms for C sequestration in the open field may negatively affect environmental safety and ecosystem security. Therefore, preventing potential risks caused by engineered biosystems through biocontainment and safety measures (e.g., prevention of unintended gene-flow or genetic modification) is crucial.

Existing technologies such as biochar, no-till farming, cover cropping, and conversion of annual crops to perennial crops have shown great potential for enhancing biocarbon sequestration. Biosystems design could be used to further improve these technologies (e.g., increasing stress tolerance in cover crops, accelerating annual-to-perennial crop conversion using synthetic biology approaches). The adoption of engineered biosystems for C sequestration is limited by several challenges, including the diversity of soil ecosystems, soil C saturation, and validating and monitoring the efficiency of long-term C sequestration. New capabilities will be needed to address these challenges, such as field-test sites that encompass a range of land types, accelerated DBTL cycles for plants, noninvasive and rapid technologies for monitoring soil C, novel C-based biomaterials, and biomaterials characterization capabilities such as accelerated aging.

Plants

Plants capture CO_2 to produce biomass and a large array of secondary metabolites. A fraction of this C is transferred to soils under the form of root exudates during plant development and via accumulation of detritus upon senescence. Engineering plants to yield more biomass and promote the deposition of stable forms of organic C in soils has the potential to mitigate increasing atmospheric CO_2 concentrations.

All plants use the Calvin–Benson–Bassham (CBB) cycle to fix CO_2 into biomass. The central enzyme in this cycle, ribulose biphosphate carboxylase (RuBisCo), is slow and exhibits both oxygenase and carboxylase activities. Furthermore, the CBB cycle requires high levels of NAD(P)H and ATP to function. To mitigate the oxygenase bypass of RuBisCo, plants have developed photorespiration pathways to recycle the products of RuBisCo oxygenase activity. Certain plants have also developed C-concentrating mechanisms to deliver low concentrations of CO_2 from air to the vicinity of RuBisCo. These mechanisms include the C_4 pathway in tropical grasses and the Crassulacean acid metabolism in arid plants. Thus, harnessing C-concentrating mechanisms and improving the efficiency of the CBB cycle offer great potential to increase CO_2 uptake and conversion. Several approaches can be considered to redesign CO_2 fixation and achieve higher plant biomass productivity. Research opportunities include

- Developing biodesigns for installation of C concentrating mechanisms in crops that do not possess them naturally;
- Improving the kinetics of RuBisCo and eliminating or reducing its oxygenase activity;
- Ameliorating the overall CBB cycle by diverting intermediates in the pathway through reactions that consume less ATP and NAD(P)H;
- Enhancing photosynthetic efficiency via synthetic photorespiratory bypass; and
- Enhancing plant C use efficiency through optimized plant architecture and reduced luxury respiration of non-photosynthetic tissues.

The ability to divert intermediates in CO_2 fixation and redesign intrinsic metabolic pathways are essential for developing strategies to generate valuable bioproducts in planta. Plants can be engineered to produce commodity chemicals and precursors for polymers that can be incorporated into building materials for long-term C sequestration (e.g., C fibers and polyurethanes). Plant bioproducts for biomanufacturing can be accumulated in specific plant tissue from which they can be easily extracted. Successful bioproduct generation from CO_2 in plants will require advances in plant biosystems design, including engineering of plant metabolic pathways and synthetic biology tools for accumulating bioproducts at economically relevant titers in desired tissues.

Crops that develop deeper root systems deposit organic C in soil horizons that have reduced microbial activity. Therefore, opportunities to input larger amounts into long–residence time SOC pools, directly sequestering CO_2 fixed through photosynthesis, include modifying root system architecture and root chemical composition. Progress in synthetic biology applied to metabolic engineering can be leveraged for the root-specific fine-tuning of polymers with extended residence time in soils, such as lignin and suberin. Similarly, new plant engineering opportunities include creating biodesigns to enrich root exudates in molecules that stably adsorb to soil minerals or become catabolized with high C use efficiency by soil microbes. The effect of redesigned root systems on the fate of plant-derived C in soils remains difficult to anticipate. The use of ^{13}C isotope-labeling methods combined with fabricated ecosystems will enable the building and testing of simulation models that predict optimal root traits and the persistent forms of plant C in soils.

Microbes

One of the main goals in engineering microbes for biocarbon sequestration is to increase stable, C-rich pathways in microbes for C sequestration and conversion at industrially relevant scales while also addressing microbial containment by restricting the ecological range of engineered strains in the environment. Although our understanding of microbial systems for C conversion and biomanufacturing continues to advance, microbial C conversion is often inefficient, and feasible bioproducts are limited and often lack performance or cost advantages to be economical. Major engineering challenges include the ability to optimize C uptake under natural conditions, inhibiting microbial respiration, the availability of advanced genetic tools for metabolic engineering, and scale-up limitations. National labs can address these challenges with interdisciplinary teams to rapidly advance microbial DBTL engineering approaches for soil C capture and capabilities for process integration and piloting. Unique capabilities at the labs—including gas fermentation scale-up (such as the Advanced Biofuels and Bioproducts Process Development Unit at LBNL), high-tech equipment to allow experiments across scales; increased automation and specialized engineering, and phenotyping with a focus on data organization and ontology to enable AI/ML with big data for predictive engineering—accelerate our ability to understand and more effectively engineer these microbial systems. With these advances, researchers can successfully engineer microbes with improved C uptake and conversion at industrially relevant scales, and develop microbial amendments that are beneficial for crop health and C sequestration.

Symbiosis (plant–microbe interaction)

The natural beneficial symbiosis can be enhanced through bioengineering of plants and microbes. Plant bioengineering to enhance symbiosis includes modification of root structure, change in the composition of exudates, genetic manipulation of key plant genes regulating symbiosis, and construction of synthetic signaling pathways to extend the host range of microbes. Microbial bioengineering to enhance symbiosis can involve engineering of effector proteins and construction of synthetic microbial communities (e.g., appropriate combination of mycorrhizal fungi and helper bacteria) that can increase host root colonization.

There are two major challenges for engineering symbiosis: a lack of efficient methods for phenotyping of roots and associated microbiomes, and limited knowledge on the fate of C in exudates and soils. To address these challenges, new capabilities need to be developed, such as improved understanding of the chemical composition of soil C and their residence times, fabricated ecosystems to test the effect of engineered plant–microbial symbiosis on C sequestration, and in vivo bioimaging systems for tracking signal transduction and metabolite/nutrient exchange at the root–microbial interface.

The outcomes to be delivered from engineering of plant–microbial symbiosis include field-tested plants and microbial systems that are ready for deployment, estimates of residence times of C molecules in soils, demonstrated region-agnostic sensor systems for C sequestration that can be paired to dynamic response systems, and predictive understanding of soil C dynamics and controls.

Breakout Group 3: Modeling and Economics

Overview

Mathematical models to elucidate and track C fluxes are necessary for valuation and optimization of biocarbon sequestration. Two types of models are widely used in biological and environmental sciences: process-based models that connect, in a causal framework, processes and conditions to outcomes or future states; and machine learning models, using predictions based on prior observations. In both cases, model parameters and relationships between parameters and outcomes are studied to determine causes and effects (e.g., to study counterfactual scenarios about future climate conditions).

Workshop participants discussed current challenges to the development and implementation of both types of models for understanding terrestrial C fluxes. Only an infinitesimal portion of the metabolic landscape that leads to terrestrial C fates is known; therefore, forward models, such as MEND (microbial-enzyme decomposition), COMETS (Computation of Microbial Ecosystems in Time and Space), EcoSys, DayCent, and DNDC (DeNitrification-DeComposition), are underspecified. Additionally, few sites are sufficiently instrumented over sufficient periods to provide data to train machine learning models. Furthermore, many forward models include parameters that are unknown, or even unknowable, with current sensing technology. For example, DayCent requests a partitioning of soil C pools into active, passive, and recalcitrant C pools at various soil depths, but these are conceptual and unmeasurable pools of SOC. When the soil microbiome changes, chemical species may undergo passive or recalcitrant changes. However, these are not intrinsic properties of chemical species, but rather, transient states determined by the present context of the soil microbiome. This level of detail is rarely if ever known about a site, so such quantities are often treated as (nearly) free parameters (i.e., values that can be tuned to fit data). However, the conventions of cross-validation and generalization errors, which are endemic to the data science community, are often not possible to contextualize because of a lack of high-quality, long-term measurements of SOC response to different treatments. This lack of standardized validation significantly reduces model reliability and must be addressed.

The convention of fitting models without rigorous checks on generalization errors is likely a primary cause of failures to translate outside of the narrow spatiotemporal domains on which such models are fitted. Indeed, AI models in key examples have now outperformed mechanistic models as measured by generalization to novel spatiotemporal domains. For modeling to progress, forward models need to be subjected to the same stringent validation requirements as AI models, and AI models must yield mechanistic insights and be applied with the express goal of discovering forward models to move science onward. Realizing these goals for validated models will require improvements in sensing, data management, and public–private collaborations.

Expanded sensing for model creation/validation at field-scale

Carbon fluxes, which are dependent on belowground processes and conditions, are difficult to manage and optimize because they are largely unquantified and invisible to farmers. Whereas crop yield and disease resistance are observable traits, root mass and SOC pools are hidden. Just as crop fields visibly transform across seasons, soil conditions also change through the year with crop growth, precipitation, freezing, thawing, tillage, and so on. These changes are further complicated because soils act as C reservoirs, making knowledge of current states necessary for determining SOC potential, and knowledge of prior conditions is beneficial in predicting future changes.

To better support modeling of belowground crop processes, expanded sensor networks that capture temporal and spatial (across fields and along the soil profile) variability are needed. One strategy could be expansion of highly instrumented fields (i.e., SMARTFARMs [Systems for Monitoring and Analytics for Renewable Transportation Fuels from Agricultural Resources and Management]) designed for ground-truthing data sets, performing deep soil sampling at high spatial resolution, and developing field-scale mass balances to thousands of acres across more regions. National labs are well poised to apply capabilities in development of low-cost sensors and remote sensing platforms to design sensor networks that enable the measurement of soil C across large areas with high spatial resolution. Partnering with the US Department of Agriculture (USDA), landowners, producers, regional academia, and industry, these expanded sensor networks across thousands of acres of row crop, pasture, and marginal lands will enable the creation of reliable large-scale data sets to support models of belowground C fluxes across the soil profile.

Data-driven integration of biological and economic models

Farm management decisions are made by predicting future states of cropping systems based on current and past conditions and projecting how these changes in crop growth and soil conditions will affect economic outcomes. As sensing technologies advance and more data are available for decision-making, improved data–model integration practices are needed to improve predictions. In particular, advances in our ability to model invisible belowground processes in the plant root zone are a limitation in accounting for C fluxes and improving soil C outcomes.

Challenges facing modeling of field-scale soil C fluxes include the need for data on current and past soil conditions and management practices, and improved models of SOC fluxes. Soil conditions and the history of land use for a property are sensitive ownership information, and C markets, which are needed for economic incentives for sustainable practices, will require data standardization and transparency. Many property owners may have competing desires support C markets and protect soil data to avoid unwanted oversight and maintain a competitive advantage. Data management standards that employ FAIR (Findability, Accessibility, Interoperability, and Reusability) practices with consideration of property owner concerns are needed to establish C markets and support large-scale models for optimizing C sequestration. From there, data repositories that can span decades, or even longer, across changes in property ownership can be developed to support identification of regions and soils that are amenable for long-term sequestration. Reliable, accessible soil data sets with improved resolution across space and time will enable the development of XAI models with uncertainty quantification. Integration of field studies with those performed in fabricated ecosystems can help provide new insights into the soil C fluxes for model refinement. These efforts will advance quantitative predictions of how SOC residence time varies across different soil conditions and will improve our understanding of long-term stability of SOC biomolecules.

The projects relevant to this report will produce extensive data sets that often get lost in typical data management systems because existing systems were not built for this scale of data. For large-scale modeling, a data/computing infrastructure is needed to facilitate FAIR representation of data with a standardized data model and provenance that easily integrates with national lab computing capabilities.

National lab capabilities in high-performance computing, large-scale data management, and model–data integration are key national resources for development of data centers to assemble large, transparent, open-access SOC data sets to support modeling of bioengineering of root–microbe–soil interactions for enhanced sustainability and economic outcomes. Prior experience in ground-truthing data sets at scale, over time and in conjunction with regional partners, and development of robust, large-scale XAI models are needed to elucidate hidden belowground processes that significantly affect crop yield and C sequestration. Through collaboration with the USDA and regional academic and industrial partners, an accessible and recognizable source can be developed for foundational ground-truthed longitudinal soil C data sets (e.g., CDIAC [Carbon Dioxide Information Analysis Center]/Ameriflux for SOC and efforts by the National Resources Inventory [NRI] and Forest Inventory and Analysis [FIA] programs), recorded alongside farming practices and weather trends.

Public–private partnerships for improved soil C management

To make management decisions to increase C sequestration (not only crop yield), growers must be able to visualize field C fluxes and must be incentivized to store C. These outcomes are best accomplished by public–private partnerships, and such collaborations are critical to balance societal sustainability goals with private business goals.

Because long-term soil health is beneficial for crop production, farmers are incentivized to choose proven sustainable practices when they have the data necessary to support these decisions. Farmers need accessible tools and applications to synthesize real-time, raw data from their fields into data-driven models to predict changes in soil C based on potential management scenarios for decision-making. Public–private partnerships are necessary at multiple stages along the development of these tools. Researchers from academia and government agencies can collaborate with producers and practitioners to instrument farms and track C fluxes to create the data needed by model developers. These modelers will then iterate with C market managers to create C valuation systems, and with agriculture industries and producers to support landowner innovations in management. Improved mechanisms for partnership and collaboration among these stakeholders for widespread instrumentation, model development, protection of data quality and ownership, and resolution of tensions between data privacy and transparency are needed to develop the modeling tools necessary for implementing improved C management at scale. These efforts will contribute to widespread, fact-based C markets that function with consumer and public trust.

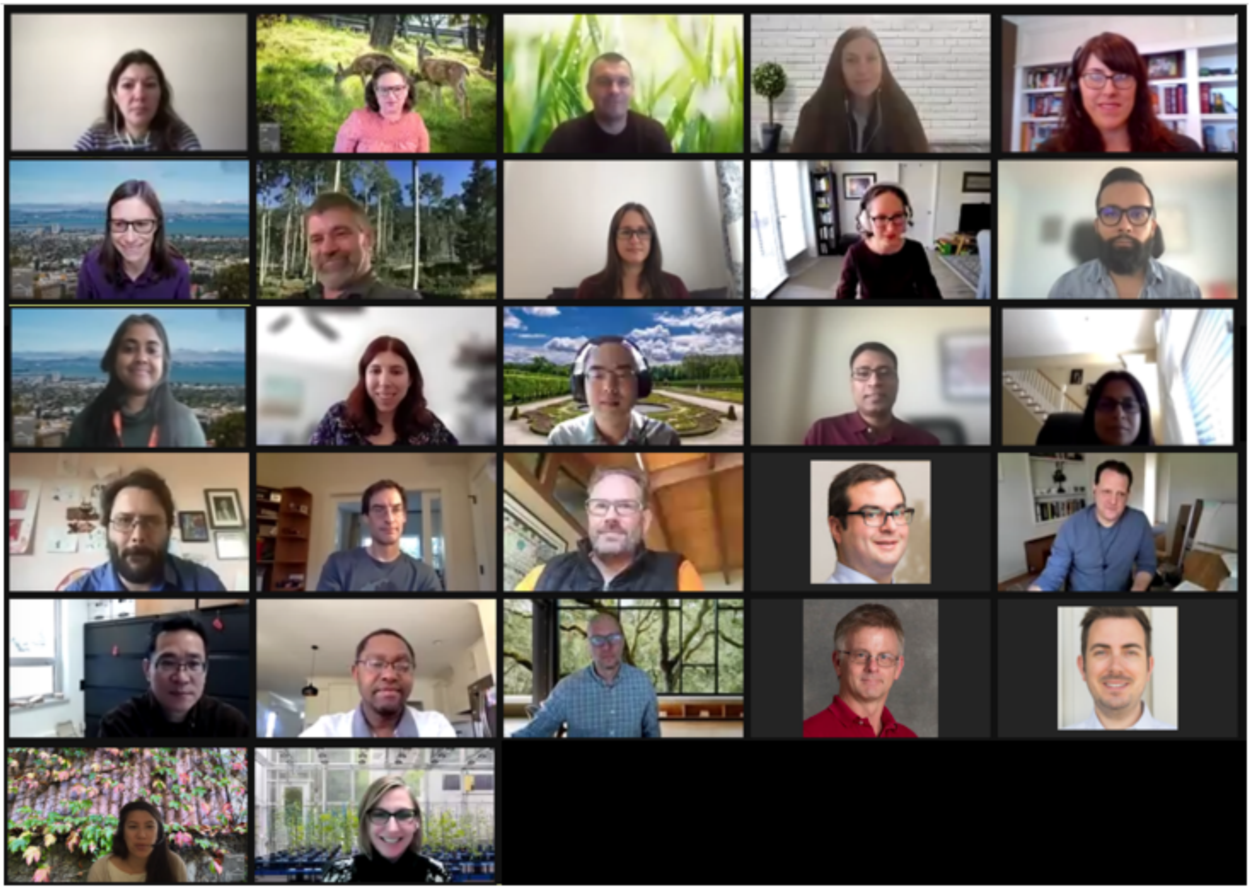
Appendix A. Abbreviations

AI/ML	artificial intelligence/machine learning
CBB	Calvin–Benson–Bassham
DBTL	design, build, test, learn
LBNL	Lawrence Berkeley National Laboratory
ORNL	Oak Ridge National Laboratory
SOC	soil organic carbon
XAI	explainable artificial intelligence

Appendix B. Workshop Participants

Table of Workshop Participants

Name	Affiliation
Ben Brown	LBNL
Javier Ceja-Navarro	LBNL
Jay Chen	ORNL
Katy Christiansen	LBNL
Melissa Cregger	ORNL
Brian Davison	ORNL
Adam Deutschbauer	LBNL
Carrie Eckert	ORNL
Aymerick Eudes	LBNL
Lauren Jabusch	LBNL
Dan Jacobson	ORNL
Melanie Mayes	ORNL
Julie Mitchell	ORNL
Wellington Muchero	ORNL
Aindrila Mukhopadhyay	LBNL
Trent Northen	LBNL
Christopher Schadt	ORNL
Henrik Scheller	LBNL
Frederik Schultz	LBNL
Michael Schuppenhauer	LBNL
Corinne Scown	LBNL
Steve Singer	LBNL
Eric Sundstrom	LBNL
Susannah Tringe	LBNL
Jerry Tuskan	ORNL
Kalluri Udaya	ORNL
Erin Webb	ORNL
Xiaohan Yang	ORNL
Larry York	ORNL
Kate Zhalnina	LBNL



A screenshot of many workshop participants (photo: Lauren Jabusch)

Appendix C. Workshop Agenda

Biologically Driven Carbon Sequestration Workshop 2

October 19, 2021

12:00–4:00 p.m. EST, 9:00 a.m.–1:00 p.m. PST

Virtual Workshop (Zoom and Miro)

Recommended Pre-Reads:
Biological Carbon Sequestration Workshop 1 white paper

Workshop Goals:

- Team building: understanding the ORNL and LBNL research expertise in biocarbon sequestration, including commitment level and potential for collaborations
- Applying an engineering approach to idea generation and proposed science to evaluate the success of C sequestration using DBTL cycles
- Information collection on three themes: natural systems, engineered systems, and modeling and economics for biocarbon sequestration.

Topic	Time
Welcome, *Julie Mitchell and Susannah Tringe*	12:00–12:10 p.m. EST 9:00–9:10 a.m. PST (10 min)
Science Talks Life Cycle Analysis and Techno-Economic Analysis for Biocarbon Sequestration, *Corinne Scown* Plant Science for Biocarbon Sequestration, *Jerry Tuskan*	12:10–12:35 p.m. EST 9:10–9:35 a.m. PST (25 min)
Introduction to Miro and practice exercise C sequestration: targets, obstacles, and measurement, *Lauren Jabusch*	12:35–1:00 p.m. EST 9:35–10:00 a.m. PST (25 min)
Break	1:00–1:10 p.m. EST 10:00–10:10 a.m. PST (10 min)
Discussion 1: Ideation on Biocarbon Sequestration Themes Introduction, *Katy Christiansen* Three tracks: • Breakout Group 1: Natural systems, *Kate Zhalnina, Julie Mitchell*	1:10–2:15 p.m. EST 10:10–11:15 a.m. PST (65 min)

Topic	Time
• Breakout Group 2: Engineered systems, *Xiaohan Yang, Aymerick Eudes* • Breakout Group 3: Modeling and economics, *Erin Webb, Lauren Jabusch*	
Break	2:15–2:25 p.m. EST 11:15–11:25 a.m. PST (10 min)
Synthesis and report back, *Group Representative(s)*	2:25–2:55 p.m. EST 11:25–11:55 a.m. PST (30 min)
Discussion 2: Round-robin review and amendment • Group 1→ Group 2 • Group 2→ Group 3 • Group 3→ Group 1	2:55–3:10 p.m. EST 11:55 a.m.–12:10 p.m. PST (15 min)
Discussion 3: Round-robin review and amendment • Group 1→ Group 3 • Group 2→ Group 1 • Group 3→ Group 2	3:10–3:25 p.m. EST 12:10–12:25 p.m. PST (15 min)
Synthesis and report back, *Group Representative(s)*	3:25–3:55 p.m. EST 12:25–12:55 p.m. PST (30 min)
Closing and next steps, *Julie Mitchell and Susannah Tringe*	3:55–4:00 p.m. EST 12:55–1:00 p.m. PST (5 min)

Organizing Committee:

Aymerick Eudes, Deputy Director, Cell Wall Biology & Engineering Group, Joint Bioenergy Institute and Scientist, Environmental Genomics and Systems Biology Division, Biosciences Area, LBNL

Erin Webb, Senior R&D Engineer and Group Leader, Bioresource Science & Engineering Group, ORNL

Kateryna Zhalnina, Research Scientist, Environmental Genomics and Systems Biology Division, Biosciences Area, LBNL

Julie Mitchell, Division Director, Biosciences Division, ORNL

Katy Christiansen, Area Science Deputy and Head, Strategic Program Development Group, Biosciences Area, LBNL

Lauren Jabusch, Program Developer, Strategic Program Development Group, Biosciences Area, LBNL

Xiaohan Yang, Senior Scientist, Synthetic Biology Group, Biosciences Division, ORNL