

Frontiers and opportunities in bioenergy crop microbiome research networks

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47 **Abstract:** Researchers from across the four U.S. Department of Energy Bioenergy Research
48 Centers engaged in a microbiome workshop that focused on identifying challenges and
49 collaboration opportunities to better understand bioenergy-relevant plant–microbe interactions.
50 The virtual workshop included hands-on educational sessions and a keynote address on current
51 best practices in microbiome science and community microbiome standards, as well as
52 breakout sessions aimed at identifying microbiome-related data and measurements that should
53 be prioritized, opportunities for and barriers to integrating plant metabolites to microbiome
54 research, and strategies for more effectively integrating microbiome data and processes into
55 existing models. Based on participant discussion, key findings of the workshop were the need
56 to prioritize scaling data sharing across BRCs and the broader research community and
57 securing collaborative infrastructure in the areas of microbiome-ecosystem modeling and
58 molecular plant-microbe interactions. This workshop review highlights additional main
59 findings from this event, to encourage cross-site and more holistic meta-analyses while
60 promoting wide scientific community engagement across plant microbiome sciences.

61

62 **Introduction**

63 Plants and microbes form a myriad of symbiotic associations, from mutualistic to
64 opportunistic to parasitic. It has become clear with recent research that many of the functions
65 of plant-associated microbes benefit the host, for example, by promoting tolerance to abiotic
66 stress, priming the immune system, or by regulating nutrient and resource availability ([Turner
67 et al. 2013](#)). Therefore, as plant - microbiome research matures, it is expected that controlling
68 or directing the plant microbiome to desired outcomes will become a key tool for crop
69 management and supporting food security and crop resilience goals ([Busby et al. 2017](#)) .

70 Bioenergy crops, also called biofuel feedstocks, are crops grown explicitly for the
71 purpose of converting plant biomass into biofuels and/or bioproducts ([Naik et al. 2010](#)). These
72 include first generation food crops such as sorghum, corn, rapeseed, and sugarcane, as well as
73 second generation cellulosic crops including switchgrass, miscanthus, alfalfa, napier grass, and
74 poplar ([Mohr and Raman 2013](#)). Third generation crops are more recently gaining interest.
75 These include boreal plants, crassulacean acid metabolism (CAM) plants, eucalyptus, and
76 microalgae. The microbiome of many of these bioenergy crops has been characterized and, in
77 some cases, linked to functionality or plant performance (e.g., ([Li et al. 2016](#))).

78 Beneficial crop microbiomes can benefit diverse agroecosystems; however, the goals
79 of bioenergy agriculture are markedly different from food agriculture. In food crop agriculture,
80 a primary objective is to increase yields on prime agricultural land, making outputs available
81 for consumption by humans and animals. Bioenergy crop agriculture shares a similar objective
82 of high biomass yield but differs in its focus on utilizing land that is marginal or of low value
83 for crop agriculture to avoid competition with food production. Additionally, there are several
84 other important sustainability objectives that are imperative for the large-scale development of
85 bioenergy crops ([Raschke et al. 2021](#)) (**Figure 1**). These objectives include limiting agricultural

86 greenhouse gas (GHG) emissions and achieving net carbon (C)-neutral or C-negative feedstock
87 production through soil C sequestration ([Gelfand et al. 2020](#)), producing bioenergy crops on
88 marginal land that is less suitable for food crops ([Solomon 2010](#)), and maintaining a neutral or
89 beneficial environmental footprint within the agroecosystem ([Robertson et al. 2017](#)) (**Box 1**).
90 It is expected that crop microbiomes will support these interrelated sustainability objectives of
91 biofuel feedstock production ([Zhalnina et al. 2021](#)), which are essential, yet not exclusive, to
92 bioenergy crops.

93 GHG emissions reduction, carbon sequestration, and environmental remediation are
94 beneficial sustainability goals for agroecosystems and have been emphasized as research
95 priorities within United States Department of Energy (US DOE)-funded bioenergy research
96 centers (BRCs). BRCs are tasked with generating data to support feedstock selection,
97 identifying and understanding impacts of land choice for feedstock cultivation, and developing
98 management strategies for bioenergy systems. Research at BRCs directly impacts the choice
99 and implementation of feedstocks and thus BRCs are uniquely poised to inform sustainable
100 development at the initial stages of management. The BRCs' roles in developing novel
101 agroecosystems set us apart from food crop agriculture, which often has had long-term
102 management practices in place. For example, the objective to grow bioenergy crops on
103 marginal lands includes research that measures nutrient and water inputs and outputs important
104 to environmental sustainability as well as economic profitability. Based on our knowledge of
105 the role of plant-microbe interactions in other systems, we predict that learning to leverage the
106 plant microbiome to benefit the host and landscape will be a decisive factor in the ultimate
107 success of bioenergy feedstocks.

108 The research priorities of lowering feedstock costs and improving year-round feedstock
109 supplies are globally relevant. In 2019, the world spent \$8B (USD) on biofuel and biogas
110 compared to the \$53B estimated to be necessary to meet in the International Energy Agency's

Sustainable Development Scenario (World Energy Investment, 2020). Thus, it is important to share BRC research priorities and impacts broadly and to maintain tight coordination and synergy between researchers within BRCs and our international scientific partners. Researchers interested in plant microbiome ecology and fundamental biological mechanisms are needed to bridge their complementary mindsets to address these grand challenges in biofuel production systems.

A workshop was organized to discuss the needs, challenges, and aspirations of plant-microbiome research in support of these distinctive objectives in bioenergy crop agriculture and to set the stage to enable and support large-scale collaborations among the four US-based BRCs (**Box 2**) and to produce a communication to share with other researchers who work in this arena. DOE BRCs are large, multidisciplinary centers spread across the United States with a focus on addressing grand challenges in biofuel research and accelerating transformational advances needed to enable cost effective and sustainable production of cellulosic biofuels in the United States (Peters 2018). The workshop was held virtually on 12 February, 2021 and attended by 74 people who represented all four BRCs and all levels of researchers, from student to investigator. Here, we report the outcomes of the sessions and discussions and aim to fuel momentum in this critical arena of feedstock microbiome research.

Workshop Description

The workshop was divided into two main sessions. The first session focused on current models of data management strategies that enable successful collaboration, followed by a keynote presentation on the National Microbiome Data Collaborative (NMDC) highlighting ongoing efforts to expand the state of the science with accessible and standardized microbiome datasets, how the data needs of the community could be coordinated, and the vision of the future NMDC data ecosystem. The second session was focused on sharing perspectives to prioritize research opportunities for collaboration in these areas.

The aim of the first session was to provide training and discussion on data sharing within large collaborations. This session was entitled “Discussion and tutorial on best practices for computational analyses and data sharing,” and included two discussions based on uniting BRC data and collaborative opportunities. Guidelines were based on, “FAIR guiding principles for scientific data management and stewardship” (Wilkinson et al. 2016), and recommendations based on performing computational analysis of large microbiome datasets (Wilkinson et al. 2016; Shade and Teal 2015; Shade et al. 2019). The first discussion focused on how research groups may make data findable, accessible, interoperable, and reusable (FAIR), specifically highlighting how these integration principles can be used throughout the data generation and publication processes. A systematic review of large microbiome datasets and their capacity to protect raw digital data, organize raw digital data, organize digital data protocols and analysis workflows, and make all digital data public and available was provided. This effort highlighted the challenge of locating deposited sequence and metabolite data (and metadata) from published studies and emphasized the challenge and collective need to consider standards for data sharing and management beyond individual labs for future collective needs. This discussion was followed by a tutorial that introduced tools for version-controlled data and protocol sharing, specifically using the GitHub platform. The objective of this tutorial was to expand the previous discussion of data hygiene and opportunities for data and analysis standardization by offering practical, concrete examples. Discussion included how to effectively reproduce previously published figures, along with the opportunities for integrating with existing collaborative tools. Subsequent discussion focused on opportunities for collaborative BRC microbiome research to leverage the NMDC.

The second session consisted of break-out group discussions on three topic areas that represent challenges to BRC microbiome research, which were selected based on survey answers from participants prior to the workshop. These challenges were: 1) priorities and

standards for collaborative microbiome-related data and measurements, 2) improved integration of plant metabolites to microbiome research, and 3) linking microbiome data and processes into existing models used within the BRCs. Below, we summarize the state of the challenges within BRC microbiome research and key opportunities for future research based on workshop discussions.

BRC Challenge 1: Priorities and standards for microbiome-related data and measurements

The tools and measurements for characterizing BRC feedstock microbiomes are similar to those used more broadly to understand plant-microbe interactions, as recently reviewed (Trivedi et al. 2021). These measurements provide insights into: 1) the plant-associated microbial communities and their functions and interactions with the plant host genotype, 2) traits and performance, and 3) ecosystem and environmental contexts. Standard microbiome measurements often include the identification and relative quantification of microbial taxa, including but not limited to bacteria, archaea, fungi, and viruses, as well as assessment or prediction of their bulk activities and functions. Such characterizations are often done for aboveground tissues including the phyllosphere but are often extended belowground to root and soil microbiome niches (Cregger et al. 2018). For the plant hosts, BRC feedstocks include productive and wild-type varieties, as well as genotypes improved through selective breeding or engineered for traits of productivity, resilience, specialized metabolism or ease of deconstruction and biofuel conversion (Hao et al. 2021; Stefani et al. 2009; Belide et al. 2017). Standard plant measurements often include the plant host genotype and host phenotypic variation across traits such as biomass, height, leaf area, biomass conversion efficiency, or disease resistance. As cellulosic biomass is often a key goal for BRC feedstocks, yield and conversion are important measurements. The management history of a field or feedstock also

influences its performance and, especially in younger fields, the documentation of stand age and environmental context can be important (Ong et al. 2016). Management factors including fertilization and other crop inputs such as bioinoculants, pesticides, water, tillage, and crop rotation history are also useful towards understanding the crop environment and their relationships to yield or sustainability objectives (Ma et al. 2021). Expanding this information and its relationships to soil and environmental characteristics such as pH, moisture, temperature, salinity, landscape/field legacy effects, and C and nitrogen (N) turnover are grand challenges in developing sustainable bioenergy cropping systems. Given the trade-offs and co-optimization among the sustainability, ecosystem services (land sharing), and yield (land sparing) objectives of agriculture (Anderson-Teixeira et al. 2012), measurements spanning environment, plant, and management are required.

Currently, there are few standardized field and plant measurement methods that are shared across BRC efforts, but there are numerous overlapping data types and needs. The differences in methodology and foci for measurements is partly due to the diversity of research questions, allocation of resources within each center, and individual investigator expertise and interest. In fact, a key realization from the workshop was the need and opportunity for BRCs to prioritize a small but impactful number of standard measurements that could broadly link microbiome and plant outcomes (e.g., plant productivity, feedstock conversion).

Recommendation 1.1: Develop a standard set of soil/environment, host, and microbiome measurements supported by cross-BRC research objectives

Given the breadth of interests across BRCs, our discussions emphasized the need to prioritize a set of soil/environment, host, and microbiome measurements (e.g., cultivation-independent and -dependent, biomass, growth stage, metabolite, biological activity rates) that can support multiple research objectives and lend themselves to inter-site comparisons and meta-analyses. Given the common objectives across BRCs to improve feedstock yield and

sustainability, any set of standard measurements should emphasize plant–microbe interactions that are associated with these objectives, including resilience (e.g., to drought), fertilizer needs (response to N inputs and limitations), and direct plant–microbe interactions (e.g., mediated by plant and microbial metabolites). A panel of key plant, environment/soil, and microbial measurements could be proposed that would be cross-cutting for plant microbiome research, including that of bioenergy crops.

There also is a need to develop and deploy both digital infrastructure to readily share standardized data and resources across BRCs, and physical infrastructure to support large-scale, cross-site experiments. For example, standardized plant-microcosms could provide tractable, comparative experimental systems for inter-BRC experiments (**Box 3**) and common field sites or experimental designs could provide cross-center insights and advances. BRCs contribute plant, fungal, and microbial genomic resources for the broader community. These rich genomic resources are available through the Joint Genome Institute database systems as well as others (IMG/ER, MycoCosm, GOLD, NCBI). A BRC-specific system to share and place these genomic data in the context of BRC experiments and, in particular, connections to plant host data and integration between plant and microbial datasets is currently lacking.

Recommendation 1.2: Develop collaborative infrastructure to directly link soil microbiomes, plant hosts, and feedstock productivity

BRCs are situated to take advantage of recently developed tools and approaches that have the potential to enhance our understanding of complex plant and soil microbiome relationships under the context of bioenergy research. There are several infrastructural advantages among the BRCs, including access to the geographic breadth of multi-site, large-scale (often long-term) field experiments with shared crops and also access to the core facilities of the Department of Energy to support sequencing, chemical, and environmental analyses of both microbes and host plants to allow for the use of cutting-edge interdisciplinary approaches

and tools. The shared mission among the BRCs of sustainable development and enhanced performance of biofuel feedstocks provides a clear connection for collaboration. Financial support through the DOE and the BRCs for workshops, working groups, meetings, and cross-invitations to seminars further enhances BRCs opportunities for engagement. Thus, the resources, scale, and technology access are aligned to support BRC efforts to advance plant microbiome understanding.

For example, maintaining and distributing microbial culture collections is a necessary but resource intensive activity that is beyond the capabilities of any one research group, and yet it is expected that sharing isolates across BRCs will be a key activity in the development and deployment of beneficial microbial consortia for biofuel feedstocks. Establishing a standard isolate-to-collection workflow, expected timeline, and accountability mechanism across BRCs to deposit microbial isolates to existing culture resources (that are equipped to handle, maintain, and make available these isolates to other researchers) would support strain resource sharing. The BRCs could also benefit from partnerships with central reference microbial strain repositories, such as the American Type Culture Collection (ATCC) (American Type Culture Collection 1997) or the Fungal Biodiversity Centre (CBS-KNAW). BRCs could do their part to provide resources to support existing culture collection infrastructure and could coordinate to regularly release updates of BRC-relevant depositions and associated metadata among researchers.

Other discussed tools with potential cross-BRC utility include quantitative stable isotope probing (Hungate et al. 2015), high-throughput cell sorting (Hatzenpichler et al. 2020), and novel culture-dependent techniques (Jo et al. 2021; Molina-Menor et al. 2020). Tools that measure plant physiological traits in real-time such as microchips (Pagay et al. 2014) and at large scales such as remote sensing using hyperspectral cameras mounted on UAVs (Li et al. 2020), aircraft, and satellites may also guide researchers to decipher factors

that drive plant performance and provide meaningful information to integrate with other current measurements. Standardized fabricated ecosystems (e.g., EcoFABS - see Box 3) provide opportunities for more controlled cross-investigator mechanistic microbiome measurements. Computational frameworks, such as artificial intelligence (AI) implementation in high resolution time-series analysis ([Nauta et al. 2019](#); [Coenen et al. 2020](#)), may be used to generate computational causal inferences, investigate how the microbiome relates to bioenergy crop phenotype, and improve our ability to navigate the large datasets we currently have, such as the ‘omics.

Since their inception in 2007, DOE BRCs have produced informative plant microbiome research through the development of large-scale collaborations and open-source resources. These include multi-omic datasets for targeted feedstocks such as poplar and switchgrass, and their associated microbiome members, including mycorrhizal fungi and plant-associated bacteria ([Martin and Bonito 2012](#); [Brown et al. 2012](#)). Identifying a set of common, impactful questions that can be addressed across bioenergy crops and centers continues to provide insight into how to leverage the biofuel crop microbiome to achieve yield and sustainability objectives and advance beyond what could be achieved in location- or crop-specific research programs. Strategies to standardize microbiome, plant, and environmental measurements, share field sites and experimental approaches, deposit and share BRC microbial isolates, and collectively adopt cutting-edge technology will foster project integration and data reuse.

BRC Challenge 2: Integration of plant metabolites to microbiome research

To understand the role of the soil and plant microbiomes on sustainable production of bioenergy crops, we need to study these interactions in these systems from the perspective of both the microbiome and the plant. Bioenergy microbiomes can directly or indirectly regulate plant metabolism ([Pang et al. 2021](#)), and similarly plant metabolites can influence the

composition and function of microbiomes in above- and below-ground tissues. Consequently, microbiome research in the BRCs requires the integration of plant and microbial metabolites to understand these interactions. The rapid development of metabolomics protocols and platforms currently provides new opportunities and challenges for advancing the elucidation of plant-microbiome interactions, including signaling, substrate exchange, and utilization.

There is a wide array of mass spectrometry (MS)- and nuclear magnetic resonance spectroscopy (NMR)-based analytical platforms that are suitable for generating broad spectrum, untargeted metabolomic profiles for characterizing the metabolites of plants and the microbial associates that constitute their microbiome. Although these platforms vary in their dynamic range, mass accuracy and range of detection, sensitivity and speed of analyses, and chromatographic resolution, they each have their utility in metabolomics. There is no single analytical platform that captures the breadth of plant and microbial metabolomes, but the application of different platforms is complementary, ensuring a greater breadth of coverage. Currently, reference metabolite databases are constrained by limited references, annotation errors due to co-eluting isobaric metabolites (i.e., metabolites whose molecular ions have identical mass and hence elemental composition), and the need for multiple chromatographic separations and ionization modes to obtain a greater coverage of the metabolome (Xiao et al. 2012). Employing complementary approaches greatly empowers these platforms, including the ability to conduct MS/MS experiments for increasing fragmentation of parent ions to inform m/z identity, and even coupling NMR for structural elucidation of unknowns. The rapid development and widespread availability of MS analytical platforms for metabolomics, including metabolite database expansion and compilation on websites that allow searchable metabolite queries, set the stage for major advances for characterizing plant-microbiome interactions.

309 The major challenges to comprehensive metabolite profiling across BRCs include the
310 large number of unannotated metabolites, difficulty in attributing metabolites to the correct
311 organismal partner in mixed communities, challenge of deciphering the metabolic exchange
312 between interacting partners, and elucidation of metabolically complex plant defense signaling
313 cascades needed to distinguish microbial friend from foe to enable symbiosis or induce defense
314 responses. A cross-institutional approach plus sharing genetic and genomic resources can
315 address these current constraints. Despite the lack of available commercial standards, many
316 labs have developed robust internal, user-defined databases that can be brought to bear on
317 a given critical unidentified metabolite. Many unknowns are designated as “known unknowns”,
318 having been observed as responsive in previous analyses and potentially by several research
319 groups. Sharing the fragmentation patterns of critical unidentified metabolites between
320 research groups can close that information gap (Wang et al. 2016) or at least identify high-
321 value unknowns.

322 Systems biology has already had a tremendous role in contributing to the elucidation
323 of unknowns, as highlighted by interrogation of microbial gene sequences for known
324 biosynthetic pathways using antibiotics and secondary metabolite analysis to identify gene
325 products that are potentially present given the presence and activity of specific microorganisms
326 (Medema et al. 2011). Additionally, multi-omic network models that include an integrated
327 metabolomics data layer associated with single nucleotide polymorphism (SNP) variation can
328 provide clues to the identity of unknown metabolites (Weighill et al. 2018, 2019). Such a multi-
329 omic network model generated for one species can inform metabolite identification for other
330 species if there is a high degree of homology between the species with respect to biosynthetic
331 pathways. Although a metabolite may be categorized as an unknown, much information on its
332 identity can still be derived from its mass to charge ratio that can be used to generate
333 hypothetical identities and support subsequent targets for synthesis and subsequent

confirmation. Furthermore, sorting out where and by whom a metabolite signal is generated can also be informed by a systems biology approach. For example, succinic acid accumulation when the fungal ectomycorrhizal symbiont *Laccaria bicolor* is associated with *Populus trichocarpa* roots is likely to be driven by the fungus degrading the plant's aromatic metabolites, as inferred from the upregulation of fungal transcripts of the pathway enzymes required to conduct the catabolism (Tschaplinski et al. 2014). Therefore, coupling MS databases, systems biology databases, and network analyses provides a powerful set of tools and approaches that can inform the underlying mechanisms driving plant–microbiome interactions.

Initiatives such as NMDC highlight the need for standardized metadata to draw meaningful conclusions from microbiome studies (Vangay et al. 2021). Integrated multi-omics approaches also need to incorporate data standards to enhance science reproducibility and results comparison across BRCs. Establishing best practices, including standards for integrated dataset collection could minimize variation in key steps during the data generation process including sampling, processing, and analytical strategies. In plant–microbe metabolomics research, we recognized that describing the plant developmental stage, how the sample is collected and stored, and which analytical tools are used during the study must be reported accurately.

Recommendation 2: Support BRC collaboration within a standard, tractable, and representative ecosystem

An opportunity for inter-site collaboration was identified in using fabricated ecosystems to address diverse questions across BRCs. Such fabricated ecosystems ('mesocosms') provide both an opportunity to increase the control, reproducibility, replicability, and observability of plant phenotypes and plant-microbe ecological interactions (Box 3). Standardized fabricated ecosystems (EcoFABs, <https://doi.org/10.1111/nph.15662>)

or other types of “rhizoboxes” can provide opportunities to: 1) enable scientists to benchmark, replicate, and build on each other’s results across BRCs, 2) create standardized data sets suitable for machine learning and other types of meta-analyses, and 3) harness advanced technologies (e.g., genetic, genomic, and metabolomic) to determine causal mechanisms underlying plant and microbial interactions.

BRC Challenge 3: Linking microbiome data and processes into existing BRC models

Mathematical models provide a way to integrate data and guide engineering efforts towards a desired outcome. Within the BRCs, microbial activity is represented in two distinct types of modeling approaches employed to determine the productivity and sustainability of bioenergy systems. First, ecosystem models are used to understand bioenergy crop yields and the environmental impact and performance of these feedstocks (e.g., soil C sequestration, N use efficiency, etc.). Second, biomass conversion models are used to study the efficacy of microbially-mediated biological conversion of plant biomass to biofuels and valuable products. The results of these two types of modeling approaches can then be integrated with techno-economic assessment (TEA) and life-cycle assessment (LCA) accounting frameworks to estimate the total costs and cradle-to-grave environmental impacts, respectively, of bioenergy production.

Process-based ecosystem models such as EPIC ([Zhang et al. 2010](#)), DayCent ([Field et al. 2018](#)), DNDC ([Brandes et al. 2018](#)), and SWAT ([Jager et al. 2015](#)) are also widely used in bioenergy crop assessment to interpret and generalize limited, discrete field measurements across space and time. Such models aim to predict energy crop yields across the heterogeneous soils, climates, and land use histories of agricultural landscapes, including lands with marginal productivity for conventional crops ([Qin et al. 2015](#)) and aim to enhance understanding of feedstock production economics and total life cycle impacts of biofuel production. Ecosystem

models can also predict how biofeedstock production impacts microbially driven processes including changes in soil C storage, emissions of nitrous oxide and other agricultural greenhouse gases, emissions of ammonia and other air pollutants, leaching of nitrate and other water pollutants, and evapotranspiration and hydrological impacts. The widely used ecosystem models listed above generally rely on conceptually-defined soil carbon pools and semi-empirical representation of soil microbial processes, lacking specificity on microbial biomass, taxonomy, and functional characteristics ([Berardi et al. 2020](#); [Campbell et al. 2018](#)).

There is widespread effort to expand these existing models or create new models that reflect an updated understanding of soil organic matter stabilization mechanisms ([Lehmann & Kleber 2015](#)) and more explicit representation of microbial biomass, functional grouping of microbial species, and microbial activity. Models such as MEND ([Wang et al. 2013](#)), CORPSE ([Sulman et al. 2014](#)), and MEMS ([Robertson et al. 2019](#)) are built around physically-measurable soil carbon pools and include dynamic microbial pools based on biomass and functional potential that influence soil processes such as decomposition rates. Parameterizing such models is a fundamental challenge ([Berardi et al. 2020](#)), but that becomes more tractable as spatially-explicit data on microbial communities becomes more available world-wide ([Crowther et al. 2019](#)).

In the process of converting cellulosic biomass into valuable bioproducts, mathematical models have been successfully used to guide bioengineering efforts to increase production. For example, genome-scale models embody a comprehensive list of metabolic processes and have been used to recommend gene knockouts that increase overall yield ([Fowler et. al 2020](#); [Maia et al. 2015](#); [Otero et al. 2013](#); [Xu et al. 2011](#)). Further, ^{13}C metabolic flux analysis can be used to obtain an insightful description of metabolic fluxes inside a cell, and this knowledge has been leveraged to identify engineering targets leading to improved production ([Chowdhury et al. 2014](#); [Costella and Martin 2018](#); [Foster et al. 2021](#); [Khodayari and Maranas 2016](#)). Kinetic

models provide a dynamic picture of metabolism as a function of time that can be used to effectively guide engineering. Furthermore, data-driven processes based on machine learning algorithms (Lawson et al. 2021) have also helped design metabolic pathways which increased productivity (Jervis et al. 2019; Radivojević et al. 2020; Zhou et al 2018). These models are contingent on large datasets. Thus, standardized data collection and sharing across BRCs would advance these research efforts.

Recommendation 3: Strategic data collection within model ecosystems

Despite the use of modelling in the production of lignocellulosic biofuels, significant challenges remain. This type of sustainability analysis provides a strong anchor point for collaboration across the BRCs and broader community. Standardized data collection through time for use in models was identified as a potential synergistic action across BRCs. In the case of ecosystem models, at minimum, microbial biomass data should be collected during soil sampling and laboratory analysis. Specific sampling and analysis protocols will vary depending on the models adopted. For example, a microbially-explicit version of DayCent in development will differentiate microbial biomass in the litter layer versus the soil (Berardi et al. 2020), whereas CORPSE differentiates between the rhizosphere and bulk soil (Sulman et al. 2014) and MEND explicitly represents microbial function via incorporation of microbial enzymatic data (Wang et al. 2013). Such data collection complements ongoing efforts to supplement soil carbon analysis with physical fractionation that separates specific organic matter pools such as particulate organic matter and mineral-associated organic matter (Lavallee et al. 2020). Further, bottom-up controlled microcosm experiments using plant hosts with defined microbial communities can provide a suitable intermediate for linking small-scale microbial ecology to larger-scale ecosystem function. Such work also has direct relevance for engineering growth-promoting microbial inoculum, a goal of some BRCs.

Conclusions

During the past decade, DOE BRCs have made significant contributions to the science, technology, and resources needed for efficient conversion of lignocellulosic feedstocks to biofuels, totaling over 3600 publications since 2007. However, sustainably produced and profitable biofuel production systems still require further development and research. A convergence of fundamental understanding of plant and microbial biochemistry, genomics, and ecology will accelerate progress toward the identification and utilization of key plant determinants and traits that drive adaptive microbiome activities and underlying plant health, yield, composition, sustainability, and resilience.

In conclusion, this workshop highlighted the need for cohesive standard data management for successful collaboration, as well as improved integration of plant and microbiome workflows across BRCs which link microbiome data and processes into existing BRC models and across the plant microbiome community. Thus, thinking systematically, collaboratively, and interactively will best leverage BRC research expertise and capabilities to tackle bioenergy and sustainability challenges. Notably, investments in the proposed key priorities should also provide data, tools, and models that can be leveraged by other researchers in the field.

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References

- American Type Culture Collection. 1997. *ATCC QC and Reference Strains*. Available at: https://books.google.com/books/about/ATCC_QC_and_Reference_Strains.html?hl=&id=PCZrAAAAMAAJ.
- Anderson-Teixeira, K. J., Duval, B. D., Long, S. P., and DeLucia, E. H. 2012. Biofuels on the landscape: Is “land sharing” preferable to “land sparing”? *Ecological Applications*. 22:2035–2048 Available at: <http://dx.doi.org/10.1890/12-0711.1>.
- Belide, S., Vanhercke, T., Petrie, J. R., and Singh, S. P. 2017. Robust genetic transformation of sorghum (*Sorghum bicolor* L.) using differentiating embryogenic callus induced from immature embryos. *Plant Methods*. 13 Available at: <http://dx.doi.org/10.1186/s13007-017-0260-9>.
- Berardi, D., Brzostek, E., Blanc-Betes, E., Davison, B., DeLucia, E. H., Hartman, M. D., Kent, J., Parton, W. J., Saha, D., & Hudiburg, T. W. 2020. 21st-century biogeochemical modeling: Challenges for Century-based models and where do we go from here? *GCB Bioenergy*, 12(10), 774–788. <https://doi.org/10.1111/gcbb.12730>
- Brandes, E., McNunn, G. S., Schulte, L. A., Muth, D. J., VanLoocke, A., and Heaton, E. A. 2018. Targeted subfield switchgrass integration could improve the farm economy, water quality, and bioenergy feedstock production. *GCB Bioenergy*. 10:199–212 Available at: <http://dx.doi.org/10.1111/gcbb.12481>.
- Brown, S. D., Utturkar, S. M., Klingeman, D. M., Johnson, C. M., Martin, S. L., Land, M. L., et al. 2012. Twenty-one genome sequences from *Pseudomonas* species and 19 genome sequences from diverse bacteria isolated from the rhizosphere and endosphere of *Populus deltoides*. *J. Bacteriol.* 194:5991–5993.
- Busby, P. E., Soman, C., Wagner, M. R., Friesen, M. L., Kremer, J., Bennett, A., et al. 2017. Research priorities for harnessing plant microbiomes in sustainable agriculture. *PLoS Biol.* 15:e2001793.
- Campbell, E. E., Field, J. L., and Paustian, K. 2018. Modelling soil organic matter dynamics as a soil health indicator. *Burleigh Dodds Series in Agricultural Science*. :97–123 Available at: <http://dx.doi.org/10.19103/as.2017.0033.21>.
- Chowdhury, A., Zomorodi, A. R., and Maranas, C. D. 2014. k-OptForce: integrating kinetics with flux balance analysis for strain design. *PLoS Comput. Biol.* 10:e1003487.
- Coenen, A. R., Hu, S. K., Luo, E., Muratore, D., and Weitz, J. S. 2020. A primer for microbiome time-series analysis. *Front. Genet.* 11:310.

- Costello, Z., Martin, H.G. A machine learning approach to predict metabolic pathway dynamics from time-series multiomics data. *npj Syst Biol Appl* **4**, 19 (2018). <https://doi.org/10.1038/s41540-018-0054-3>.
- Cregger, M. A., Veach, A. M., Yang, Z. K., Crouch, M. J., Vilgalys, R., Tuskan, G. A., et al. 2018. The *Populus* holobiont: dissecting the effects of plant niches and genotype on the microbiome. *Microbiome*. 6:31.
- Crowther, T. W., Hoogen, J. van den, Wan, J., Mayes, M. A., Keiser, A. D., Mo, L., Averill, C., & Maynard, D. S. 2019. The global soil community and its influence on biogeochemistry. *Science*, 365(6455), eaav0550. <https://doi.org/10.1126/science.aav0550>
- d'Espaux, L., Ghosh, A., Runguphan, W., Wehrs, M., Xu, F., Konzock, O., et al. 2017. Engineering high-level production of fatty alcohols by *Saccharomyces cerevisiae* from lignocellulosic feedstocks. *Metab. Eng.* 42:115–125.
- Field, J. L., Evans, S. G., Marx, E., Easter, M., Adler, P. R., Dinh, T., et al. 2018. High-resolution techno-ecological modelling of a bioenergy landscape to identify climate mitigation opportunities in cellulosic ethanol production. *Nature Energy*. 3:211–219 Available at: <http://dx.doi.org/10.1038/s41560-018-0088-1>.
- Foster, C.J., Wang, L., Dinh, H., Suthers, P.F., and Maranas, C. 2021. Building kinetic models for metabolic engineering. *Current Opinion in Biotechnology*, 67:35-41, <https://doi.org/10.1016/j.copbio.2020.11.010>.
- Fowler, Z. L., Gikandi, W. W., and Koffas, M. A. G. 2009. Increased malonyl coenzyme A biosynthesis by tuning the *Escherichia coli* metabolic network and its application to flavanone production. *Appl. Environ. Microbiol.* 75:5831–5839.
- Gelfand, I., Hamilton, S. K., Kravchenko, A. N., Jackson, R. D., Thelen, K. D., and Robertson, G. P. 2020. Empirical evidence for the potential climate benefits of decarbonizing light vehicle transport in the u.s. with bioenergy from purpose-grown biomass with and without BECCS. *Environ. Sci. Technol.* 54:2961–2974.
- Ghosh, A., Ando, D., Gin, J., Runguphan, W., Denby, C., Wang, G., et al. 2016. ¹³C metabolic flux analysis for systematic metabolic engineering of *S. cerevisiae* for overproduction of fatty acids. *Frontiers in Bioengineering and Biotechnology*. 4 Available at: <http://dx.doi.org/10.3389/fbioe.2016.00076>.
- Hao, Z., Yogiswara, S., Wei, T., Benites, V. T., Sinha, A., Wang, G., et al. 2021. Expression of a bacterial 3-dehydroshikimate dehydratase (QsuB) reduces lignin and improves biomass saccharification efficiency in switchgrass (*Panicum virgatum* L.). *BMC Plant Biol.* 21:56.
- Hatzenpichler, R., Krukenberg, V., Spietz, R. L., and Jay, Z. J. 2020. Next-generation physiology approaches to study microbiome function at single cell level. *Nat. Rev. Microbiol.* 18:241–256.

- 552 Hungate, B. A., Mau, R. L., Schwartz, E., Caporaso, J. G., Dijkstra, P., van Gestel, N., et al.
 553 2015. Quantitative microbial ecology through stable isotope probing. *Appl. Environ.*
 554 *Microbiol.* 81:7570–7581.
 555
- 556 Jager, H. I., Baskaran, L. M., Peter E. Schweizer, Turhollow, A. F., Brandt, C. C., and
 557 Srinivasan, R. 2015. Forecasting changes in water quality in rivers associated with growing
 558 biofuels in the Arkansas-White-Red river drainage, USA. *GCB Bioenergy*. 7:774–784
 559 Available at: <http://dx.doi.org/10.1111/gcbb.12169>.
 560
- 561 Jervis, A., Carbonell, P., Vinaixa, M., Dunstan, M., Hollywood, K., Robinson, C., Rattray,
 562 N., Yan, C., Swainston, N., Currin, A., Sung, R., Toogood, H., Taylor, S., Faulon, J.,
 563 Breitling, R., Takano, E., and Scrutton, N. 2019. Machine learning of designed translational
 564 control allows predictive pathway optimization in *Escherichia coli*. *ACS Synth. Biol.*, 8:127–
 565 136. <https://doi.org/10.1021/acssynbio.8b00398>
 566
- 567 Jo, C., Bernstein, D. B., Vaisman, N., Frydman, H. M., and Segrè, D. 2021. A co-culture
 568 microplate for real-time measurement of microbial interactions. *bioRxiv*. Available at:
 569 <http://biorxiv.org/lookup/doi/10.1101/2021.01.07.425753>.
 570
- 571 Khodayari, A., Maranas, C. A genome-scale *Escherichia coli* kinetic metabolic model k-
 572 ecoli457 satisfying flux data for multiple mutant strains. *Nat Commun* 7, 13806 (2016).
 573 <https://doi.org/10.1038/ncomms13806>.
 574
- 575 Lavalley, J. M., Soong, J. L., & Cotrufo, M. F. 2020. Conceptualizing soil organic matter into
 576 particulate and mineral-associated forms to address global change in the 21st century. *Global*
 577 *Change Biology*, 26(1), 261–273. <https://doi.org/10.1111/gcb.14859>
 578
- 579 Lawson, C. E., Martí, J. M., Radivojevic, T., Jonnalagadda, S. V. R., Gentz, R., Hillson, N.
 580 J., et al. 2021. Machine learning for metabolic engineering: A review. *Metabolic*
 581 *Engineering*. 63:34–60 Available at: <http://dx.doi.org/10.1016/j.ymben.2020.10.005>.
 582
- 583 Lehmann, J., & Kleber, M. 2015. The contentious nature of soil organic matter. *Nature*.
 584 <https://doi.org/10.1038/nature16069>
 585
- 586 Li, D., Voigt, T. B., and Kent, A. D. 2016. Plant and soil effects on bacterial communities
 587 associated with *Miscanthus giganteus* rhizosphere and rhizomes. *GCB Bioenergy*. 8:183–193
 588 Available at: <http://dx.doi.org/10.1111/gcbb.12252>.
 589
- 590 Li, F., Piasecki, C., Millwood, R. J., Wolfe, B., Mazarei, M., and Stewart, C. N., Jr. 2020.
 591 High-throughput switchgrass phenotyping and biomass modeling by UAV. *Front. Plant Sci.*
 592 11:574073.
 593
- 594 Ma, L. ⁺, Rocha, F., Lee, J., Choi, J., Tejera, M., Sooka-Nguan, T., Boersma, N., VanLoocke,
 595 A., Heaton, E., and Howe, A. 2021. The impact of stand age and fertilization of the soil
 596 microbiome of *Miscanthus x giganteus*. *Phytobiomes Journal*.
 597 <https://doi.org/10.1094/PBIOMES-01-20-0014-FI>.
 598
- 599 Maia, P., Rocha, M., and Rocha, I. 2016. *In silico* constraint-based strain optimization
 600 methods: the quest for optimal cell factories. *Microbiol. Mol. Biol. Rev.* 80:45–67.
 601

- Martin, F., and Bonito, G. 2012. Ten years of genomics for ectomycorrhizal fungi: What have we achieved and where are we heading? In *Edible Ectomycorrhizal Mushrooms*, Soil Biology, Springer Berlin Heidelberg, p. 383–401.
- Medema, M. H., Blin, K., Cimermancic, P., de Jager, V., Zakrzewski, P., Fischbach, M. A., et al. 2011. antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids Res.* 39:W339–46.
- Mohr, A., and Raman, S. 2013. Lessons from first generation biofuels and implications for the sustainability appraisal of second generation biofuels. *Energy Policy.* 63:114–122 Available at: <http://dx.doi.org/10.1016/j.enpol.2013.08.033>.
- Molina-Menor, E., Gimeno-Valero, H., Pascual, J., Peretó, J., and Porcar, M. 2020. High culturable bacterial diversity from a European desert: The Tabernas Desert. *Front. Microbiol.* 11:583120.
- Naik, S. N., Goud, V. V., Rout, P. K., and Dalai, A. K. 2010. Production of first and second generation biofuels: A comprehensive review. *Renewable and Sustainable Energy Reviews.* 14:578–597 Available at: <http://dx.doi.org/10.1016/j.rser.2009.10.003>.
- Nauta, M., Bucur, D., and Seifert, C. 2019. Causal discovery with attention-based convolutional neural networks. *Mach. Learn. Knowl. Extr.* 1:312–340.
- Okahashi, N., Matsuda, F., Yoshikawa, K., Shirai, T., Matsumoto, Y., Wada, M., et al. 2017. Metabolic engineering of isopropyl alcohol-producing *Escherichia coli* strains with C-metabolic flux analysis. *Biotechnol. Bioeng.* 114:2782–2793.
- Ong, R. G., Higbee, A., Bottoms, S., Dickinson, Q., Xie, D., Smith, S. A., et al. 2016. Inhibition of microbial biofuel production in drought-stressed switchgrass hydrolysate. *Biotechnol. Biofuels.* 9:237.
- Otero, J. M., Cimini, D., Patil, K. R., Poulsen, S. G., Olsson, L., and Nielsen, J. 2013. Industrial systems biology of *Saccharomyces cerevisiae* enables novel succinic acid cell factory. *PLoS One.* 8:e54144.
- Pagay, V., Santiago, M., Sessoms, D. A., Huber, E. J., Vincent, O., Pharkya, A., et al. 2014. A microtensiometer capable of measuring water potentials below -10 MPa. *Lab Chip.* 14:2806–2817.
- Pang, Z., Chen, J., Wang, T., Gao, C., Li, Z., Guo, L., et al. 2021. Linking plant secondary metabolites and plant microbiomes: A review. *Front. Plant Sci.* 12:621276.
- Peters, N. K. 2018. U.S. Department of Energy Bioenergy Research Centers: 10-Year Retrospective. Breakthroughs and Impacts, 2007-2017. Available at: <http://dx.doi.org/10.2172/1471705>.
- Qin, Z., Zhuang, Q., and Cai, X. 2015. Bioenergy crop productivity and potential climate change mitigation from marginal lands in the United States: An ecosystem modeling

- perspective. *GCB Bioenergy*. 7:1211–1221 Available at: <http://dx.doi.org/10.1111/gcbb.12212>.
- Radivojević, T., Costello, Z., Workman, K. *et al.* A machine learning Automated Recommendation Tool for synthetic biology. *Nat Commun* **11**, 4879 (2020). <https://doi.org/10.1038/s41467-020-18008-4>
- Raschke, A., Sebastian Hernandez-Suarez, J., Pouyan Nejadhashemi, A., and Deb, K. 2021. Multidimensional aspects of sustainable biofuel feedstock production. *Sustainability*. 13:1424 Available at: <http://dx.doi.org/10.3390/su13031424>.
- Robertson, A. D., Paustian, K., Ogle, S., Wallenstein, M. D., Lugato, E., & Cotrufo, M. F. (2019). Unifying soil organic matter formation and persistence frameworks: The MEMS model. *Biogeosciences*, 16(6), 1225–1248. <https://doi.org/10.5194/bg-16-1225-2019>
- Robertson, G. P., Hamilton, S. K., Barham, B. L., Dale, B. E., Izaurrealde, R. C., Jackson, R. D., *et al.* 2017. Cellulosic biofuel contributions to a sustainable energy future: Choices and outcomes. *Science*. 356 Available at: <http://dx.doi.org/10.1126/science.aal2324>.
- Sasse, J., Kant, J., Cole, B. J., Klein, A. P., Arsova, B., Schlaepfer, P., *et al.* 2019. Multilab EcoFAB study shows highly reproducible physiology and depletion of soil metabolites by a model grass. *New Phytol*. 222:1149–1160.
- Shade, A., Dunivin, T. K., Choi, J., Teal, T. K., and Howe, A. C. 2019. Strategies for building computing skills to support microbiome analysis: a five-year perspective from the EDAMAME workshop. *mSystems*. 4 Available at: <http://dx.doi.org/10.1128/mSystems.00297-19>.
- Shade, A., and Teal, T. K. 2015. Computing workflows for biologists: A roadmap. *PLoS Biol*. 13:e1002303.
- Solomon, B. D. 2010. Biofuels and sustainability. *Ann. N. Y. Acad. Sci*. 1185:119–134.
- Stefani, F. O. P., Moncalvo, J.M., Séguin, A., Bérubé, J. A., and Hamelin, R. C. 2009. Impact of an 8-year-old transgenic poplar plantation on the ectomycorrhizal fungal community. *Appl. Environ. Microbiol*. 75:7527–7536.
- Sulman, B. N., Phillips, R. P., Oishi, A. C., Shevliakova, E., & Pacala, S. W. (2014). Microbe-driven turnover offsets mineral-mediated storage of soil carbon under elevated CO₂. *Nature Climate Change*, 4(12), 1099–1102. <https://doi.org/10.1038/nclimate2436>
- Trivedi, P., Mattupalli, C., Eversole, K., and Leach, J. E. 2021. Enabling sustainable agriculture through understanding and enhancement of microbiomes. *New Phytol*. Available at: <http://dx.doi.org/10.1111/nph.17319>.
- Tschaplinski, T. J., Plett, J. M., Engle, N. L., Deveau, A., Cushman, K. C., Martin, M. Z., *et al.* 2014. *Populus trichocarpa* and *Populus deltoides* exhibit different metabolomic responses to colonization by the symbiotic fungus *Laccaria bicolor*. *Mol. Plant. Microbe. Interact*. 27:546–556.

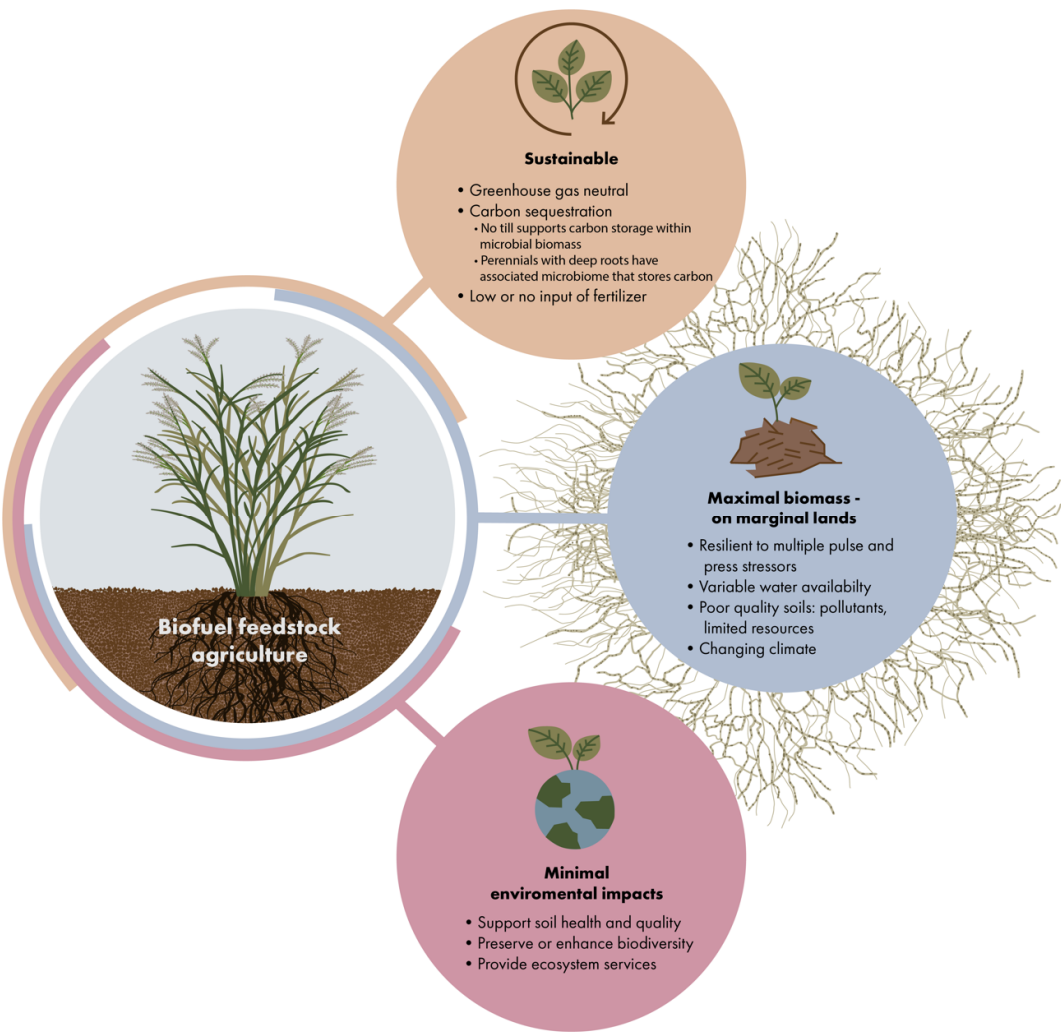
- Turner, T. R., James, E. K., and Poole, P. S. 2013. The plant microbiome. *Genome Biol.* 14 Available at: <http://dx.doi.org/Artn 209Doi 10.1186/Gb-2013-14-6-209>.
- Vangay, P., Burgin, J., Johnston, A., Beck, K. L., Berrios, D. C., Blumberg, K., et al. 2021. Microbiome metadata standards: Report of the National Microbiome Data Collaborative's workshop and follow-on activities. *mSystems.* 6 Available at: <http://dx.doi.org/10.1128/msystems.01194-20>.
- Wang, G., Post, W. M., & Mayes, M. A. (2013). Development of microbial-enzyme-mediated decomposition model parameters through steady-state and dynamic analyses. *Ecological Applications*, 23(1), 255–272. <https://doi.org/10.1890/12-0681.1>
- Wang, M., Carver, J. J., Phelan, V. V., Sanchez, L. M., Garg, N., Peng, Y., et al. 2016. Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. *Nat. Biotechnol.* 34:828–837 Available at: <http://dx.doi.org/10.1038/nbt.3597>.
- Wang, Z., Chen, Z., Kowalchuk, G. A., Xu, Z., Fu, X., and Kuramae, E. E. 2021. Succession of the resident soil microbial community in response to periodic inoculations. *Appl. Environ. Microbiol.* 87 Available at: <http://dx.doi.org/10.1128/AEM.00046-21>.
- Weighill, D., Jones, P., Bleker, C., Ranjan, P., Shah, M., Zhao, N., et al. 2019. Multi-phenotype association decomposition: Unraveling complex gene-phenotype relationships. *Front. Genet.* 10:417.
- Weighill, D., Jones, P., Shah, M., Ranjan, P., Muchero, W., Schmutz, J., et al. 2018. Pleiotropic and epistatic network-based discovery: Integrated networks for target gene discovery. *Front. Energy Res.* 6 Available at: <http://journal.frontiersin.org/article/10.3389/fenrg.2018.00030/full>.
- Wilkinson, M. D., Dumontier, M., Aalbersberg, I. J. J., Appleton, G., Axton, M., Baak, A., et al. 2016. The FAIR Guiding Principles for scientific data management and stewardship. *Sci Data.* 3:160018.
- World Energy Investment 2020. Available at: <https://www.iea.org/reports/world-energy-investment-2020> [Accessed September 15, 2021].
- Xiao, J. F., Zhou, B., and Ransom, H. W. 2012. Metabolite identification and quantitation in LC-MS/MS-based metabolomics. *Trends Analyt. Chem.* 32:1–14.
- Xu, P., Ranganathan, S., Fowler, Z. L., Maranas, C. D., and Koffas, M. A. G. 2011. Genome-scale metabolic network modeling results in minimal interventions that cooperatively force carbon flux towards malonyl-CoA. *Metab. Eng.* 13:578–587.
- Zhalnina, K., Hawkes, C., Shade, A., Firestone, M. K., and Pett-Ridge, J. 2021. Managing plant microbiomes for sustainable biofuel production. *Phytobiomes Journal.* 5:3–13 Available at: <http://dx.doi.org/10.1094/pbiomes-12-20-0090-e>.

748 Zhang, J., Petersen, S. D., Radivojevic, T., Ramirez, A., Pérez-Manríquez, A., Abeliuk, E., et
749 al. 2020. Combining mechanistic and machine learning models for predictive engineering and
750 optimization of tryptophan metabolism. *Nat. Commun.* 11:4880.
751
752 Zhang, X., Izaurrealde, R. C., Manowitz, D., West, T. O., Post, W. M., Thomson, A. M., et al.
753 2010. An integrative modeling framework to evaluate the productivity and sustainability of
754 biofuel crop production systems. *GCB Bioenergy*. 2:258–277 Available at:
755 <http://dx.doi.org/10.1111/j.1757-1707.2010.01046.x>.

756 Zhou, Y., Li, G., Dong, J., Xing, X., Dai, J., and Zhang, C. 2018. MiYA, an efficient
757 machine-learning workflow in conjunction with the YeastFab assembly strategy for
758 combinatorial optimization of heterologous metabolic pathways in *Saccharomyces*
759 *cerevisiae*. *Metabolic Engineering* 47:294-302, <https://doi.org/10.1016/j.ymben.2018.03.020>.
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Figures

Figure 1: Summary of objectives in the production of sustainable bioenergy feedstocks



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Box 1: Objectives of bioenergy feedstock agriculture

Carbon neutrality: Agricultural production is associated with greenhouse gas (GHG) emissions from the production of fertilizers and other inputs, on-farm energy use, changes in soil carbon storage, and other soil trace gas emissions. Bioenergy crop production should ideally be at least net carbon neutral (with soil carbon sequestration offsetting production of other GHGs), or, more desirably, carbon negative. Practically, this requires sustainable agricultural management practices that minimize inputs such as fertilizer and irrigation use and promote soil C sequestration by reducing or eliminating tillage and growing perennial bioenergy crops such as switchgrass, miscanthus, and poplar that allocate substantial carbon to roots and root exudates.

Marginal land utilization: Growing bioenergy crops on prime agricultural land increases costs and leads to undesirable “food versus fuel” competition which remains a key criticism of first-generation bioenergy systems-based corn, sugarcane, sorghum and other food crops. Therefore, the BRCs specifically and the bioenergy industry more generally increasingly target the production of dedicated bioenergy crops on land that is less suitable for food production, i.e., “marginal land”. While the specific definitions of marginal lands vary widely, the ability of bioenergy crops to contend with suboptimal conditions such as transient flooding, drought, poor soil quality, and possible pollutants, high temperatures, or wind is critical for lowering production costs and conflicts with existing agricultural production.

Sustainable production: Finally, minimizing the environmental impact of bioenergy crop agriculture is also a key objective. Bioenergy cropping systems should increase or preserve soil health and quality, local biodiversity, water quality, and other key ecosystem services. Environmental impact may be estimated by measuring the ecological footprint of bioenergy agroecosystems and applying a systems-level analytical approach.

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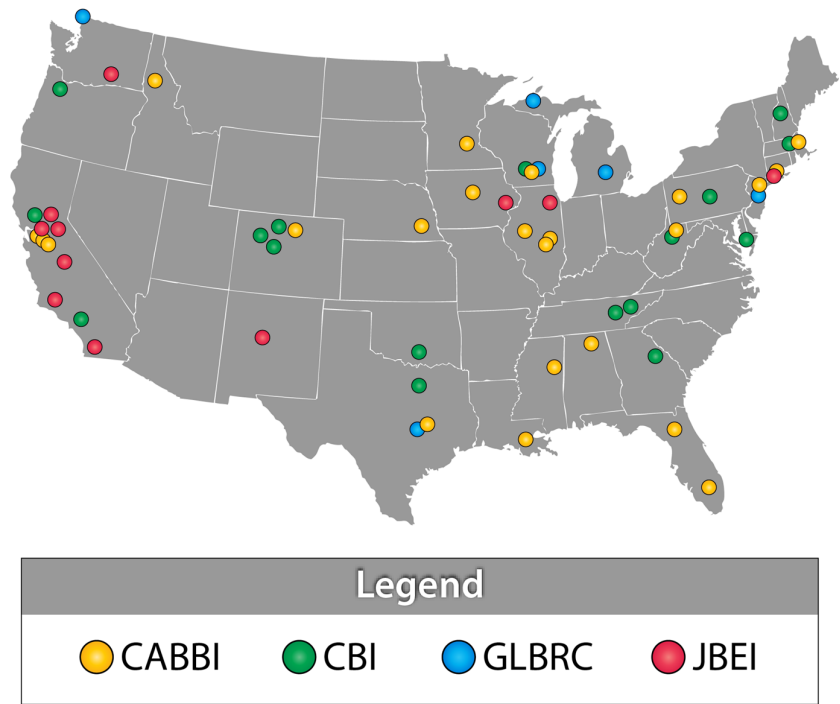
Box 2: BRC vision statements along with locations of all collaborative institutions that are associated with a specific BRC.

CABBI – Integrate recent advances in agronomics, genomics, and synthetic and computational biology to increase the value of energy crops — using a “plants as factories” approach to grow fuels and chemicals in plant stems, an automated foundry to convert biomass into valuable chemicals, and ensuring that its products are ecologically and economically sustainable.

CBI – Accelerate domestication of bioenergy-relevant plants and microbes to enable high impact, value-added fuels and coproduct development at multiple points in the bioenergy supply chain.

GLBRC – Develop sustainable biofuels and bioproducts from all usable portions of dedicated energy crops grown on marginal, nonagricultural lands.

JBEI – Convert bioenergy crops into economically-viable, carbon-neutral, biofuels and renewable chemicals currently derived from petroleum, and many other bioproducts that cannot be efficiently produced from petroleum.



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Box 3: Existing fabricated ecosystems include single plant-scale EcoFAB devices.

EcoFAB device consists of an autoclavable physical chamber (enabling gnotobiotic studies), target plant (optional), growth medium (e.g., soil, sand, media), and the microbial communities to be tested. EcoFABs are designed for ‘omics analysis and high-resolution rhizosphere imaging. The ‘1.0’ EcoFAB devices use 3-D printing to create molds for casting the biocompatible polymer polydimethylsiloxane (PDMS) into the upper portion of a fluidics chamber (Inset). This is a very flexible approach that enables rapid design alteration to address specific research questions. More recently, ‘2.0’ EcoFAB devices have been constructed using injection molding of polycarbonate. These standardized devices can be mass produced to support large-scale studies of plants and rhizosphere microbial communities. This is subsequently attached to a microscope slide, completing the chamber. Both systems can be sealed to support analysis of volatile metabolites and stable isotope probing/labeling experiments. They have been successfully used to study a diversity of plants (*Brachypodium*, *Arabidopsis*, *Meticago*, switchgrass, etc.) and microorganisms. Data collected in duplicated systems in different labs had high reproducibility ([Sasse et al. 2018](#)) suggesting that they will be suitable for inter-BRC collaborations.



Fabricated ecosystems (EcoFAB) showing *Brachypodium distachyon* growing in hydroponic solution. Image credit: Kateryna Zhalnina. Image credit: Thor Swift/Berkeley Lab.