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Research for Sustainable Bioenergy: Linking Genomic and Ecosystem Sciences

Workshop Report

U.S. DEPARTMENT OF ENERGY | Office of Science

Office of Biological and Environmental Research

Research for Sustainable Bioenergy Workshop

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Mission

The Office of Biological and Environmental Research (BER) advances world-class fundamental research programs and scientific user facilities to support the Department of Energy's energy, environment, and basic research missions. Addressing diverse and critical global challenges, the BER program seeks to understand how genomic information is translated to functional capabilities, enabling more confident redesign of microbes and plants for sustainable biofuel production, improved carbon storage, or contaminant bioremediation. BER research advances understanding of the roles of Earth's biogeochemical systems (the atmosphere, land, oceans, sea ice, and subsurface) in determining climate so that it can be predicted decades or centuries into the future, information needed to plan for energy and resource needs. Solutions to these challenges are driven by a foundation of scientific knowledge and inquiry in atmospheric chemistry and physics, ecology, biology, and biogeochemistry.

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Executive Summary

The design of sustainable biofuel systems requires knowledge about key plant-microbe-environment interactions that provide a range of ecosystem services. Most critical is a mechanistic understanding of how candidate biofuel plants interact with biotic and abiotic factors to affect the ecosystem outcomes that define sustainability. Recent advances in the genomic sciences can contribute immensely to the knowledge needed to design such systems. For example, progress in plant genomics will enable the inclusion of sustainability traits in future feedstocks, and advances in microbial genomics will allow insights into plant-microbe-soil interactions that might be used to manage and support plant productivity and vigor. Linking these advances to breakthroughs in ecosystem science enables the use of systems biology in the fundamental design of sustainable biofuel systems.

To identify research opportunities in developing such systems, the Department of Energy's (DOE) Office of Biological and Environmental Research (BER) held the Research for Sustainable Bioenergy Workshop on Oct. 2–4, 2013, in Germantown, Maryland. The workshop convened more than 30 researchers with a broad and diverse range of expertise, including ecology, microbiology, plant genetics, genomics, computational biology, and modeling. Participants discussed and identified research gaps, challenges, and opportunities for enhancing the understanding of influences that biotic, abiotic, and genetic factors have on long-term plant feedstock performance and the delivery of ecosystem services at multiple scales. This report identifies the key topics and questions that could be addressed effectively to achieve this understanding. Research opportunities are organized into four categories: (1) plant systems, encompassing plant productivity, resource use efficiency, genotype/phenotype breeding, and crop diversity; (2) the plant microbiome, which includes microbes living in close association within or adjacent to plants; (3) ecosystem processes, such as carbon capture, greenhouse gas mitigation, and hydrologic processes; and (4) multiscale modeling, which integrates and extends results across spatiotemporal scales.

- ***Plant Systems.*** In agricultural systems, plant productivity generally is limited by the availability of essential resources such as light, water, and nutrients. A plant's ability to capture and use these resources is affected by its resource use efficiency, phenology, and capacity to thrive under a wide range of environmental conditions and is a key determinant of both productivity and sustainability. All these attributes are genetically influenced and potentially could be modified to produce plants well suited for biofuel production with minimal inputs. For example, researchers have identified a number of root traits affecting water, nitrogen (N), and phosphorus (P) acquisition and can use this knowledge to enable trait-based selection of superior biofuel species genotypes. Rapid advances in DNA sequencing technology have the potential to revolutionize marker development and deployment and to accelerate the breeding of superior genotypes. High-throughput sequencing may be especially helpful for overcoming obstacles inherent in breeding polyploid outcrossing species such as switchgrass. Also important is breeding for genotype mixtures that could provide niche complementarities not available in single-genome or single-species cropping systems, especially amid growing environmental variability.

- ***The Plant Microbiome.*** Some well-known beneficial plant-associated microbes include bacteria that fix N, mycorrhizal fungi that enhance P delivery, and rhizosphere bacteria that provide growth-promoting hormones. Other associations are only surmised, and the expanding application of microbial metagenomics, metatranscriptomics, and metabolomics to plants and their immediate surroundings suggests a world of hitherto unknown associations. This knowledge is leading to a paradigm-shifting view of plants as metaorganisms rather than isolated individuals, with huge implications for plant breeding and crop management. To exploit these evolving insights, critical gaps need to be filled in current understanding of the plant microbiome. These gaps include (1) the degree of species specificity between plants and microbes (e.g., What are the associations and their functional roles, and how might they be managed?); (2) the nature of interactions between plants and surrounding rhizosphere consortia (e.g., Can specific microbial relationships enable plants to tolerate stresses ranging from nutrient and water deficiencies to pathogen attack?); and (3) the potential application of synthetic biology (*de novo* engineering of genetic circuits and the biological processes they control) to biofuel systems (e.g., Can novel beneficial relationships be established in which microbes persist inside plants, on leaf surfaces, and in the rhizosphere?).

- ***Ecosystem Processes.*** Carbon (C), N, and P cycling; greenhouse gas emissions; and the movement of water through soils and landscapes are key processes that fundamentally affect the sustainability of biofuel systems. The biogeochemical cycling of all major nutrients can be altered when existing lands are converted to bioenergy cropping systems. Interactions among plants and microbes within a particular soil-climate location will largely determine the direction, magnitude, and timing of such alterations (including losses) and will shape the new equilibrium state. Likewise, plant-microbe interactions affect greenhouse gas emissions. Each of the three major biogenic gases—carbon dioxide, nitrous oxide, and methane—contributes differentially to a cropping system's greenhouse gas balance and is emitted or consumed by different groups of soil microbes. Thus, understanding the genetic makeup and functional significance of different microbial taxa and how they are influenced by plant, edaphic, and climatic factors is key to managing biogeochemical fluxes for sustainable outcomes. Additionally, how biofuel systems affect the flow and quality of water leaving these systems has an enormous impact on groundwater and downstream ecosystems. Plants play a direct role in the hydrologic cycle via transpiration, which is affected by differing growth rates, phenologies, and root architectures; microbes play an indirect role via effects on plant vigor and stress tolerance.
- ***Multiscale Modeling.*** Multiscale models enable integration of information from multiple biological scales into a predictive understanding of sustainability outcomes across a range of spatial and temporal scales, including future climate and management scenarios. Incorporating genomic information into these models represents a new frontier with considerable promise, especially for those ecosystem processes resistant to conventional quantitative modeling. The opportunity to develop multiscale, mechanistic models is expanding as large datasets with both genomic and ecosystem information become available and as process-level functional understanding of genomic and phenomic differences among plants and their microbiomes improves.

Realizing the promise of genomic knowledge to better understand and predict ecosystem behavior will require field sites for long-term systems-level research, instrumentation to link advances in the genomic and ecosystem sciences, computational resources to provide visualization and modeling capabilities, and support for workforce training. Field sites similar to the Integrated Field Laboratories described in *BER Virtual Laboratory: Innovative Framework for Biological and Environmental Grand Challenges* (BERAC 2013) are needed to place disciplinary research in a systems context and provide opportunities for multiscale model validation. Experiments at these field sites must be evaluated over appropriate temporal scales (i.e., years to decades) to capture development trajectories for perennial crops and the influence of episodic environmental events such as climate extremes and pathogen outbreaks. Implicit in this call is the need for sustained commitment to support the long-term experiments and cyberinfrastructure that will be necessary for data analysis and multiscale modeling.

Access to new and advanced instrumentation also will be important. Existing DOE user facilities will be invaluable for many questions, but advanced field instrumentation also is necessary. Examples include (1) *in situ* soil sensors for observing roots, their exudates, and organic compounds of microbial origin; (2) *in situ* isotope imaging and other sensors that provide quantitative measures of C, N, and oxygen dynamics at microscales; and (3) coordinated phenotyping platforms that provide analyses of specific phenes across and within different soil-climate locations. Computational resources are required to organize and share heterogeneous ecological data. Additionally, new software tools are needed to process phenotypic data in the context of metadata not currently used in breeding programs, and new hardware is necessary to run high-resolution models and visualizations.

Meeting these challenges requires a workforce empowered to use a new generation of genomic technologies and computational tools coupled with advanced experimentation. Interdisciplinary training that enables scientists with deep expertise in one area to extend their knowledge to a systems context will be critical, and workforce training in plant ecophysiology, mechanistic multiscale modeling, microbial ecology, and organismic biology, among other disciplines, is needed. Teams of scientists who have interlocking expertise and a systems perspective will be key to advancing a systems-level understanding of biofuel cropping systems.

Plant feedstocks hold great promise as economically and environmentally viable alternatives to fossil fuels. However, this new bioenergy agriculture will likely impose significant changes to the crop-soil-microbe ecosystem as it currently exists, presenting unique challenges further complicated by climate variability and change. Fully understanding the potential environmental impacts of widespread biofuel agriculture will require models that accurately predict the responses of plant and microbial species to these changes. By increasing knowledge of the biological mechanisms underlying feedstock productivity, efforts in plant and plant-microbe-soil research can both advance development of next-generation bioenergy crops and provide healthy, vibrant ecosystems and associated ecosystem services. Recent advances in systems biology and genomics technologies are providing unprecedented opportunities for mapping the complex networks underlying molecular mechanisms of plant and microbial growth, development, and metabolism. These tools will be crucial for understanding the interrelationships between a fully sustainable bioenergy agricultural system and whole ecosystems.

Introduction

The emergence of a viable cellulosic bioenergy industry opens opportunities for designing biofuel feedstock systems that are substantially more sustainable than their grain-based counterparts. Properly designed cellulosic feedstock systems have the potential to avoid most of the environmental shortcomings of grain-based systems and, if implemented on lands not suitable for row crops, can avoid biofuel competition with food production. Moreover, many cellulosic systems have the capacity to deliver ecosystem services not provided by existing biofuel production systems, including conservation benefits such as endangered bird habitat and soil restoration.

Realizing these potentials is not necessarily straightforward, however. Providing climate change mitigation and improved air, soil, and water quality requires knowledge about the key players, namely the plants that supply the biomass used to produce fuels, the microbes that support plant productivity and vigor, and all other organisms and environmental factors that contribute to the sustainability of highly productive ecosystems. Crucial questions include:

- Which sustainability traits can be bred into candidate feedstocks, and how will the market value those traits?
- What are the advantages and disadvantages of monocultures versus mixed species plantings?
- Can microbes be managed to improve the success of plants grown in environments known for water, nutrient, and pest stress?
- Can systems be managed to minimize greenhouse gas production, limit the escape of reactive nitrogen and phosphorus, favor soil carbon storage, and enhance biodiversity and associated ecosystem services?
- To what extent can all systems be managed similarly, or will best management strategies vary substantially from system to system?
- How can sites and soils best suited to these uses be identified?

These questions must be addressed in a systems context because the various pieces interact in sometimes surprising ways that cannot be predicted from knowledge of individual parts. Additionally, the net effect of all interactions provides the sustainability attributes society demands (i.e., productive feedstocks grown in a way that protects and even enhances the environment). Knowledge, therefore, must be sought and applied *in situ* and at spatial scales relevant to management and environmental impact, from organismal to landscape (see Fig. 1. Multiscale Nature of Genome-Based Biofuel Sustainability, p. 2). The temporal dimension is equally important. Microbial and plant communities change through time as they establish, equilibrate, and respond to changing environmental conditions. This multiscale complexity demands a modeling context, which can be used to predict the success of systems in different environments over time. Multiscale models will be needed to quantitatively predict the productivity and environmental consequences of deploying feedstock systems in different regions. For many of these places, limited empirical knowledge exists, and nearly all locations will be subject to climate change and variability accompanied by complex interactions and consequences. These factors require reliance on process-based models to predict productivity and environmental outcomes.

Recent advances in the genomic sciences have the capacity to contribute immensely to the knowledge needed to design sustainable biofuel systems. Contributions of genomic sciences to biofuel processing and conversion technologies are well known, and applying their power to sustainability questions offers substantial promise. Advances in plant genomics enable the identification and inclusion of sustainability traits in future feedstocks. Advances in microbial genomics will provide new insights into plant-microbe-soil interactions that can be used to manage and support plant productivity and environmental quality. Linking these advances to those in ecosystem science provides an unprecedented opportunity to substantially advance both fundamental knowledge of systems biology in general as well as an enhanced, more directed ability to design sustainable biofuel systems.

Research for Sustainable Bioenergy

This report assesses the potential for bringing advances at the intersection of the genomic and ecosystem sciences to bear on the sustainability of future cellulosic cropping systems. In each of three major areas—plant systems, the plant microbiome, and ecosystem processes—candidate research topics are highlighted, along with sets of compelling questions that could be opportunely addressed to achieve such advances, in addition to a fourth research area, modeling needs. Finally, the crucial infrastructure needs required to undertake this research are identified, including the field facilities, instrumentation, computing, and workforce support not currently available.

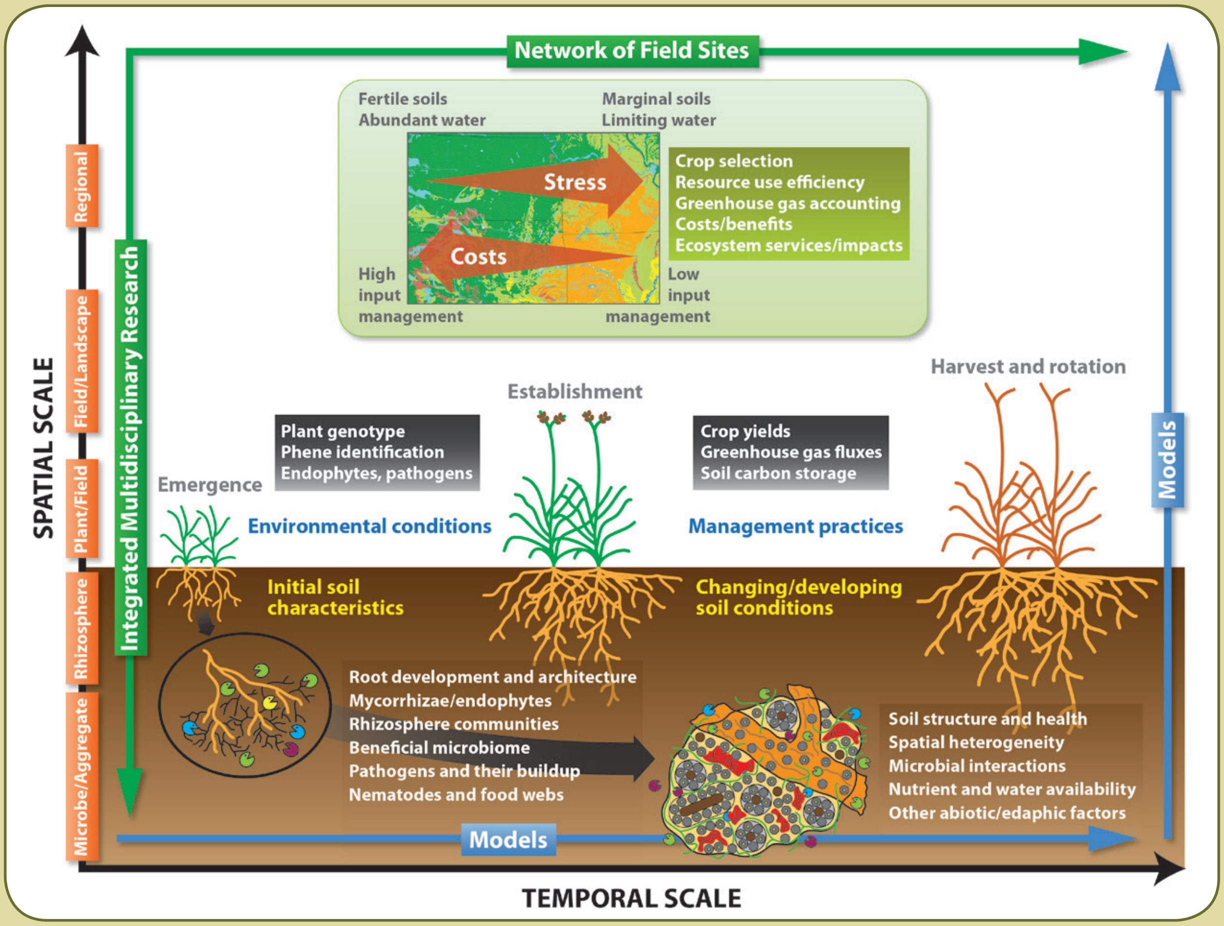

Fig. 1. Multiscale Nature of Genome-Based Biofuel Sustainability. Organisms and processes acting at local spatial scales of rhizospheres and soil particles (bottom left, y-axis) provide ecosystem services that aggregate at larger scales to ecosystem and landscape-level impacts (upper left, y-axis). Microbial and plant communities also change through time (x-axis), resulting in development trajectories that can span decades. Models allow local attributes and outcomes to be extrapolated to larger areas and projected to longer futures. [Top center green box: soil map background courtesy U.S. Department of Agriculture Natural Resources Conservation Service. Figure at bottom, to right of black arrow: soil aggregate illustration modified from Jastrow and Miller 1998.]

Main Operational Challenges

Broadly defined, there are two main operational challenges facing cellulosic biofuel production: sustainable yields and the delivery of ecosystem services other than yield. The chief challenge facing sustainable yields is the capacity for plants to be productive in the face of multiple stresses and reduced inputs—water, nutrients, and pests in particular. Because feedstocks might best be produced on nonforest land not now used to grow food (Robertson et al. 2008), this challenge is likely to be even more acute than for food crops. Typically, such lands are less fertile, receive and retain less water, and are more prone to erosion (see Fig. 2. Challenges of Marginal Soils, below). Moreover, many of the climate and other benefits that biofuels provide can be sharply compromised by management inputs with high intrinsic carbon (C) and energy costs. Sustainable yield thus requires that biofuel cultivars exhibit an enhanced capacity to use water and nutrients efficiently, acquire nitrogen and phosphorus (P) from nutrient-depleted soils, and withstand pests and disease with minimal interventions.

The delivery of ecosystem services other than yield follows from the relatively recent recognition that agricultural systems in general are capable of providing services typically associated only with natural ecosystems (Swinton et al. 2007; Power 2010). Ecosystem services are the benefits people obtain from ecosystems. Valued services include water supplies free of nitrate, dissolved P, and other contaminants; climate stabilization via greenhouse gas mitigation and C sequestration; biodiversity services such as pest regulation, enhanced pollination, and habitat suitable for threatened and endangered species (Werling et al. 2014); and aesthetic and cultural amenities such as green space and places to hunt, hike, and birdwatch. Many of these services could be readily supplied by sustainable cellulosic cropping systems.

The design of multifunctional cropping systems provides the opportunity for a number of alternative management decisions that will affect the mix of ecosystem services provided. Chief among them is crop choice. Whether the crop will be perennial or annual, native or exotic, herbaceous or woody, single or multispecies, invasive or noninvasive, each of these choices will have fundamental consequences for the delivery of specific services. Some species will provide conservation benefits, whereas others will not. Some will mitigate greenhouse gases more effectively than others. Some will provide pollinators and biocontrol agents for other crops in the landscape, but others will not. Some will be more nutrient or water efficient and better adapted to less fertile soils, and some may have microbiomes more amenable to management than others.

For a variety of reasons, a single plant species is unlikely to dominate cellulosic feedstock production, even in the United States. Research thus should encompass many different species in the quest to link plant and microbial genomes to ecosystem processes

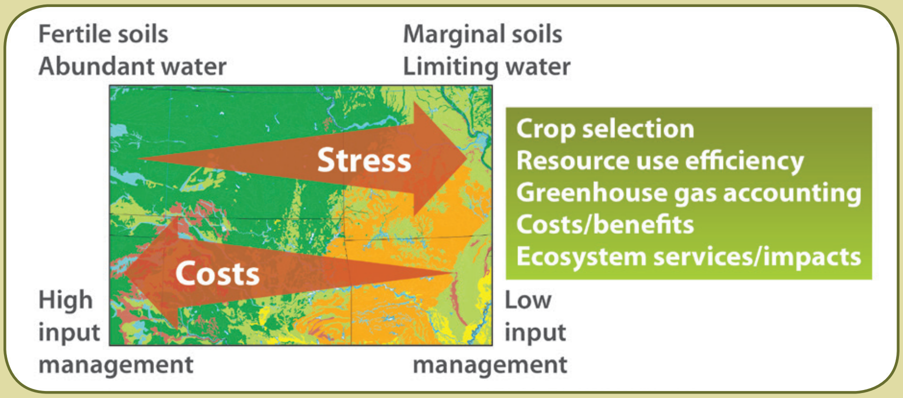

Fig. 2. Challenges of Marginal Soils. Biofuel crops planted on marginal soils with less inherent fertility face stresses to minimize management inputs that would otherwise compromise their climate and other social benefits. These stresses are exacerbated by environmental and economic imperatives. (For broader context, see Fig. 1, p. 2.)

for different soil-climate combinations. Furthermore, scientific discoveries in noncrop species can serve as models for use in future biofuel species.

The choice of perennial versus annual species also creates a special temporal dimension to feedstock production and research. Annual crops, while environmentally and economically expensive, are renewed every year, providing greater flexibility with respect to a grower's ability to rotate quickly to different crops or cultivars. Perennial crops, on the other hand, while environmentally and economically less costly, take longer to become productive. Their longer establishment phase (i.e., time to maximum production, typically taking several years) and often high planting costs can make perennials economically difficult for a producer to rotate quickly into different crops or varieties. Additionally, there may be an associated establishment phase for the microbes important to crop success. Perenniality thus provides both an operational and a research challenge, insofar as research must span years (see Fig. 1, p. 2) to include both the establishment and post-establishment phases. In fact, shortening the establishment phase might itself be a positive outcome of cellulosic biofuels research.

Research Opportunities

Research opportunities follow from the main operational challenges facing sustainable biofuel production. These opportunities are grouped into four categories, primarily for convenience, but all are interrelated and comprise parts of the same interrelated systems. The intent of this section is to highlight the most compelling issues, organizing them into logical, but not exclusive, groups.

The first group of research opportunities is plant systems, a category that includes plant productivity, resource use efficiency, genotype/phenotype breeding, and crop diversity. The second group is organized around the plant microbiome, which includes microbes living wholly within the plant (endophytes); mycorrhizal fungi, which live both within the plant and in the surrounding soil; and other microbes inhabiting the rhizosphere including rhizosphere consortia, nitrogen (N)-fixing microbes (diazotrophs), and pathogens and insect pests. Ecosystem processes, the third group of opportunities, includes carbon (C) capture and sequestration, greenhouse gas mitigation and albedo change, and hydrology and hydrologic nutrient loss. Multiscale modeling provides an important fourth research opportunity.

1. Plant Systems

Plant Productivity

Plant productivity, the ability of plants to produce useful biomass, is an essential characteristic of sustainable biofuel systems. The conversion of light energy, water, carbon dioxide (CO_2), and minerals into photosynthates (carbohydrates, reduced N, and reduced sulfur compounds) is the primary source of the material and embedded energy that are harvested to produce biofuels, as well as the primary source of sustenance for microbes and other soil biota that comprise a functional cropping system. Maintaining a high level of plant productivity is therefore essential for the ecological and economic vitality of cellulosic production systems.

Plant productivity in most agricultural ecosystems is limited by the suboptimal availability of one or more resources such as light, heat, water, or nutrients. Thus, resource use efficiency (i.e., the ability to convert system inputs into useful outputs, encompassing both acquisition and utilization efficiencies) is important for plant productivity, as addressed in the following section, p. 6. Several other plant processes also are inherently associated with potential productivity. Phenology is of overarching importance in that it defines the time period and environmental regime of plant growth and development. In general, longer annual growth periods, as well as a perennial life history, allow a plant to capture and utilize resources over a longer period, improving production. However, longer phenologies may also entail tradeoffs in some environments because the growing season might be extended into unfavorable conditions such as cold, heat, or drought. Climate change is likely to affect crop productivity in part by affecting crop phenologies, with yet unknown consequences (Leakey, Ort, and Long 2006).

Another overarching characteristic that drives plant productivity is vigor. Some genotypes of the same crop species have been commonly observed to have superior growth and yield under a range of conditions; they are more vigorous. The mechanistic basis of crop vigor is not well understood, and in some crops it includes biochemical, physiological, and developmental mechanisms, each underpinned by genetics.

Adaptation to specific environmental conditions is also a determinant of plant productivity. The most productive biofuel crops will be well adapted throughout their ecoregion. In some cases, broad adaptation can be ascribed to a specific environmental factor such as temperature, but, in many cases, the basis of this adaptation is not understood. Specialized adaptations can also be important, especially in the presence of more localized abiotic stressors such as salinity, trace element concentrations, and air pollutants such as ozone.

In addition to the overarching characteristics of phenology, vigor, and adaptation, several physiological processes are directly linked to plant productivity. They include C gain through photosynthesis, loss through respiration, and allocation to various plant tissues and the rhizosphere, as well as the partitioning of N and other resources to and from harvested plant components. Although photosynthetic C gain has been intensively researched, mechanisms controlling biomass allocation among and within plant organs, and fitness tradeoffs for contrasting allocation strategies, are still poorly understood (Flexas et al. 2006; Kakani et al. 2011). This is especially true for C allocations to roots, the rhizosphere, and microbial partners, which are critical processes in the context of agroecosystem sustainability.

The following questions represent compelling research opportunities with direct relevance for understanding and improving biofuel crop productivity:

- How can crop phenology be manipulated to maximize productivity in different environments, including future climates?
- Which genetic and physiological mechanisms—encompassing molecular, cellular, organ, and organismic processes—control crop vigor and broad adaptation?
- Which genetic and physiological mechanisms control the partitioning of photosynthates to different plant tissues, to beneficial microbial communities, and to the rhizosphere?
- At the organism and stand scale, what is the optimal partitioning of plant resources to autotrophic and heterotrophic tissues and harvestable yields in the context of long-term system productivity?

Resource Use Efficiency

Sustainable agroecosystems are capable of efficiently converting valuable inputs such as the three most common limiters of productivity—water, phosphorus (P), and N—into valued outputs with minimal waste. Resource use efficiency underpins many aspects of system sustainability, including reduced demand for external inputs and, consequently, reduced economic and energy costs. Other aspects are reductions in the generation of potentially harmful waste products and therefore reduced environmental costs. In the important case of water, improved efficiency also may make a system more drought resistant (Blum 2005). Agronomic management has important consequences for resource use efficiency at both the field and landscape scales. At the scale of individual plants, substantial intraspecific variation for resource use efficiency can be harnessed to develop more resource efficient crops.

Water use efficiency (WUE) will be increasingly important as climate change alters the spatiotemporal distribution of precipitation, higher temperatures increase evaporative losses from soils and plants, and water consumption (for both human use and crop irrigation) increases as a result of population growth and anticipated economic development. WUE has been intensively researched for many years, focusing on the transpiration efficiency (TE) of leaves and canopies, biomass partitioning between roots and aboveground parts of the plant, and inter- and intraspecific variation (Blum 2005, 2009; Clifton-Brown and Lewandowski 2000; Erickson et al. 2012). This research has produced promising tools for crop breeding. For example, variation in TE associated with water conductance in leaves may reduce water loss. Traits conferring more effective water acquisition are less understood but include improved rooting depth and optimization of soil water use over the growing season by slowing water use early in the crop cycle so that soil water is available later in the season (Singh et al. 2012). The spatiotemporal control of water uptake by roots, and water transport through root tissues, is complex and only slowly being unraveled (Schachtman and Goodger 2008; Zhang et al. 2009; Manschadi et al. 2010; Schoppach and Sadok 2012).

Increasing plant P efficiency is important because high-grade P deposits are nonrenewable and are projected to be significantly depleted in this century. Additionally, P effluents in runoff and erosion are significant sources of water pollution. Substantial intraspecific variation for P efficiency has been used to develop new cultivars of rice, common bean, and soybean with greater P use efficiency. These cultivar lines have root traits that enhance topsoil exploration. Researchers have identified a number of root traits, or "phenes" (see sidebar, Phenotyping Plants, p. 7), that enhance P acquisition. Mycorrhizal symbioses and P-solubilizing root exudates and microbes in the rhizosphere are important for P mobilization and plant P acquisition, although how these processes can be improved through crop breeding or by manipulating soil microbes is not yet clear.

Nitrogen use efficiency (NUE) is an essential component of sustainable biofuel systems because the production of N fertilizer is energy intensive, and soil N not taken up by plants or immobilized by microbes is a significant source of greenhouse gases and water pollution. Substantial intraspecific variation for NUE is evident in crop germplasm, but the mechanisms underlying this variation are poorly understood. Root traits that accelerate soil exploration in deep soil strata are useful for capturing nitrate as it leaches through the soil. Furthermore, plant associations with diazotrophic microbes could be optimized to increase NUE of bioenergy crops. In perennial plants, maximizing retranslocation to roots prior to shoot harvest is a powerful means to conserve both N and P for growth during the following growing season.

Plant phenology, including development, growing season duration, and perenniality, has important consequences for resource use efficiency by regulating resource acquisition and utilization and synchronizing crop demand with resource availability. Growing season duration and perenniality can be especially important for minimizing hydrologic N loss during the spring and fall when, in humid locations, rainfall exceeds evapotranspiration. The spatiotemporal complexity of root-soil processes calls for a robust functional-structural modeling effort to understand how root phenes affect crop performance in specific environments.

Phenotyping Plants

The term “phene” was coined in 1925 to describe phenotypic traits under genetic control (see Fig. 3. Phene Interactions, below; Serebrovsky 1925). More recently introduced is the concept: *Phene* is to *phenotype* as *gene* is to *genotype* (Lynch 2011; Pieruschka and Poorter 2012). Despite the long history of this term, however, it is rarely used in plant biology and ecology, which employ more ambiguous terms such as “trait.” Just as the development of a rich vocabulary has been instrumental in conceptualizing the diverse elements of the genome, development of more precise and specific terminology is needed to dissect and understand the diverse elements of the *phenome*.

Researchers have proposed that the most meaningful phenes are *elementary* and *unique* at their level of biological organization (Lynch and Brown 2012). For example, rooting depth is not an elementary root architectural phene since several distinct, more elementary root phenes control it. Elementary phenes should have unique developmental pathways (i.e., distinct pathways should not give rise to the same phenotype). Many measurements of plant phenotypes are aggregates of multiple elementary phenes, or *phene aggregates* (York, Nord, and Lynch 2013). Phene aggregates influence plant functional responses that in turn determine yield (see Fig. 4. Systems Approach for Biofuels Sustainability Research, p. 8). A more precise definition of the phenome’s diverse elements will afford several conceptual and practical benefits for phenome research. For example, use of the more precise term, *target phenes*, as opposed to *phene aggregates* would be useful in developing phenotyping platforms to identify genes for superior resource use efficiency or stress tolerance in crop improvement.

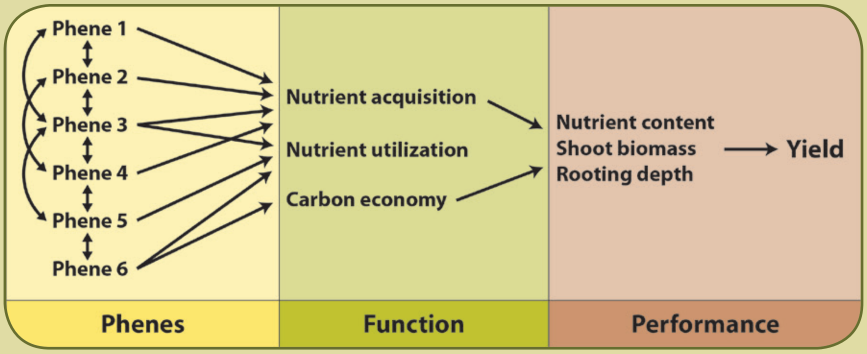

Fig. 3. Phene Interactions. Individual phenes interact to affect plant sustainability and aggregate attributes that contribute to sustainable yields.

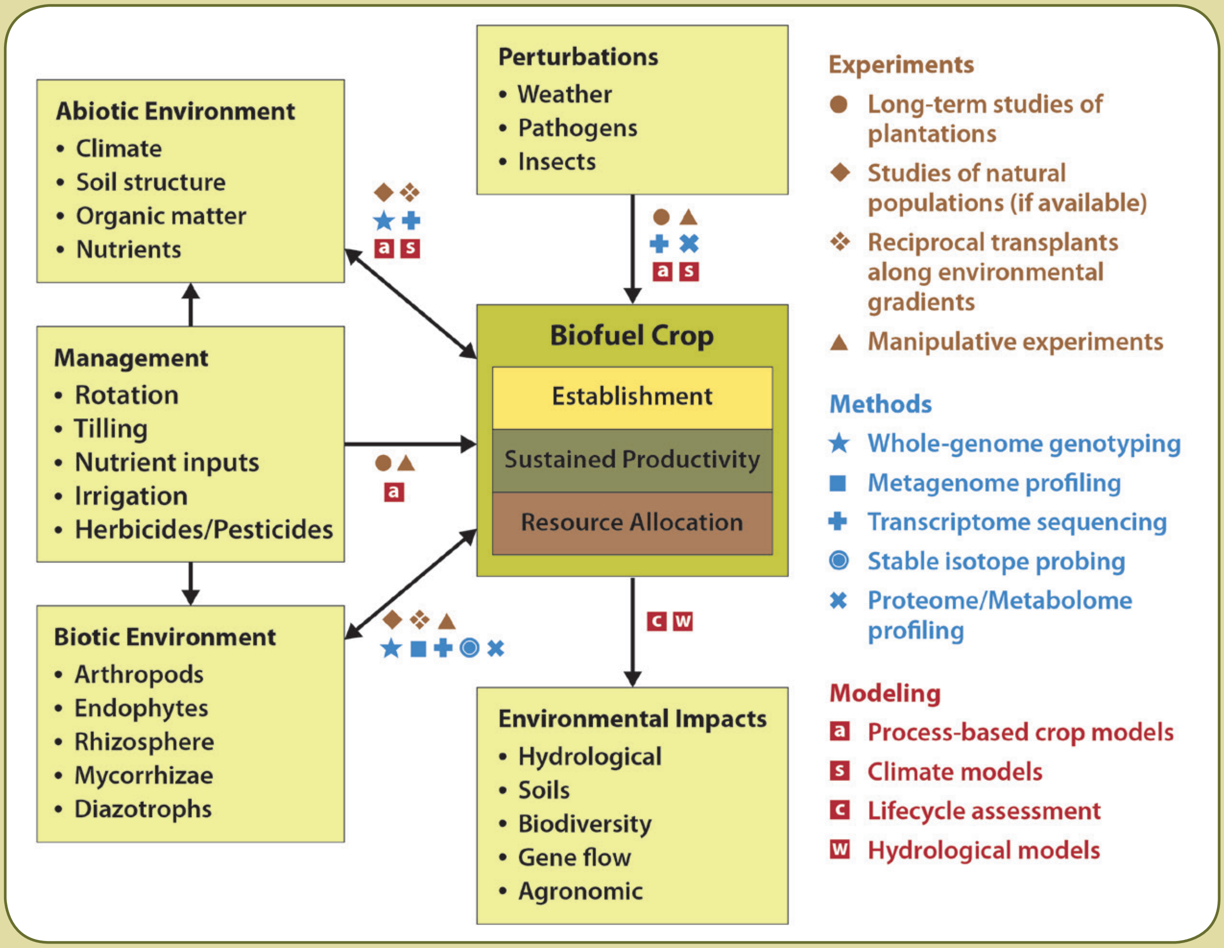

Fig. 4. Systems Approach for Biofuels Sustainability Research. Abiotic and biotic environments interact to affect the success and environmental impacts of biofuel crops in both the establishment and productivity phases, which also are affected by episodic acute perturbations such as weather events and pest outbreaks. Multiscale modeling, informed by place-based, long-term observations and experiments, enables the extension of knowledge to different environments with extrapolation to regional and global scales.

The following questions represent compelling research opportunities with direct relevance for understanding and improving the resource use efficiency of biofuel crops. They relate to the general question: Which genetic and physiological mechanisms can optimize the capture and utilization of water, P, and N over the growing season? More specific research questions include:

- Which leaf and canopy traits can be deployed to improve water utilization by feedstock species?
- Which traits can be used to improve water capture, including root penetration of drying soil, water uptake from soil, and water transport to shoots?
- Which traits can be deployed to improve P acquisition, including root architecture, root exudates, and microbial associations, for feedstock species?
- Which traits can be exploited to improve N acquisition, including traits enhancing soil exploration at depth, association with diazotrophic or other rhizosphere microbes, and traits reducing nitrification?
- Given the apparent different requirements on root architecture for maximum N versus P uptake, to what extent is it possible to optimize P and N acquisition simultaneously?

- Which shoot traits can be deployed to optimize N and P utilization in shoot tissues, especially N allocation to optimize photosynthesis and, in perennial plants, to maximize the remobilization of N and P to roots prior to harvest?

Genotype/Phenotype Breeding

Plant breeding for food and feed over the past century has focused on maximizing yield and nutrient content with moderate to high inputs of fertilizers [especially N, P, and potassium (K)], other agricultural chemicals (e.g., herbicides and pesticides), and augmented water supplies where irrigation is available. Plant breeders have paid scant attention to increased NUE *per se* (biomass yield per unit of N applied), although some crops are using less N because of breeding for lower grain protein or higher starch content. Opportunities to pursue improved resource use efficiency under low-input (or no input) conditions will become especially attractive, as will breeding for plant traits that enhance the plant microbiome, including associations with beneficial microbes both inside the plant and in its surroundings.

A shift in breeding strategy is needed to produce biofuel genotypes adapted to rainfed marginal areas in particular, where the objective is long-term biomass harvested from lands with low-nutrient and water-holding capacity soils (Gelfand et al. 2013) and with minimal to no inputs of nutrients, water, or pesticides (see Fig. 2, p. 3). Lower inputs will limit yields unless breeding and other strategies are directed toward increasing biofuel plants' ability to maximize biomass under low-input conditions. Some of the traits or phenes for nutrient and water use efficiency and tolerance to abiotic and biotic stresses are known (Sinclair 2000, 2012; Mutava et al. 2011; Lynch 2013). However, more must be discovered to enable trait-based selection of superior (e.g., high resource use efficiency) biofuel genotypes for breeding programs (Condon et al. 2004). This need is especially true for perennial grasses and trees, which have substantially longer breeding cycles relative to annual crops.

Discovering new phenes underlying productivity and yield under nutrient- and water-limited conditions may require new phenotyping tools and approaches but may also be as simple as changing the basic selection environment. Discovery of new phenes will enable identification of underlying genes and development of associated markers that can be used for DNA-based selection of superior genotypes for breeding.

Rapid advances in DNA sequencing technology and associated dramatic reductions in costs have enabled sequencing of not only reference genotypes for many crop species, but also hundreds of additional genotypes that capture much of the genetic and phenotypic diversity of some species. These genome sequence resources have revolutionized marker development and deployment and facilitated novel strategies such as genome-wide association studies (GWAS; McCarthy et al. 2008) and genomic selection (Meuwissen, Hayes, and Goddard 2001) to accelerate breeding of superior genotypes. High-throughput sequencing of genomes or subgenomes may be helpful especially for overcoming some of the obstacles inherent in breeding polyploid (e.g., tetraploid and octaploid) outcrossing species such as switchgrass and species with long generation times such as forest trees (Grattapaglia et al. 2009).

Breeding for maximal yield with minimal inputs in different climates and soils requires access to seed sources and field sites at which different plant sustainability traits can be evaluated simultaneously (e.g., see Fig. 5. Optimal Trait Selection, p. 10). Public seed banks are needed to capture the genetic diversity of multiple candidate biofuel species for research and breeding purposes. Field sites are needed for long-term genome and phenome studies. Ideally, these sites should be replicated across appropriate edaphic and environmental gradients and equipped with sensors to measure important environmental and soil attributes and processes including N, P, and water fluxes and identify traits best suited to low-input environments. Measurements of the availability of soil water and nutrients, as well as organic matter content, also will inform breeders and others about water and nutritional limitations on growth and resource use efficiencies and provide information on the delivery of potential ecosystem services by different plant genomes (see Fig. 6. Switchgrass Harvest, p. 10).

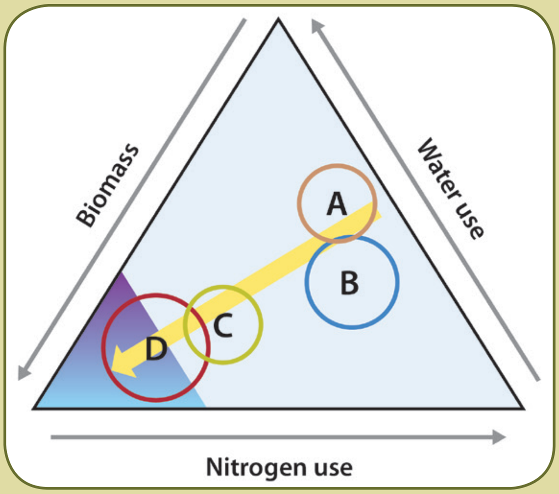

Fig. 5. Optimal Trait Selection. Simultaneously evaluating different plant sustainability traits allows breeders to evaluate species and genotypes for optimal combinations of traits. In this example, the optimal combination of traits for biomass, water use, and nitrogen use is depicted for four species A–D.

Compelling research questions for plant breeding targeted at sustainable biofuel production include:

- What are the key phenes and corresponding genes and alleles of biofuel species that confer critical traits such as nutrient and water use efficiency and tolerance to biotic and abiotic stress?
- How can genome sequences of multiple, diverse genotypes of a given target species best be used to accelerate the breeding process to achieve sustainability objectives?
- Which ecosystem services are provided by biofuel species in different environments, and how can these be valued so they are incorporated into breeding objectives?
- To what extent do microbes contribute to plant performance and/or ecosystem services, and how can positive plant-microbe interactions be maximized in cropping systems by developing cultivars that can take advantage of beneficial interactions?

Fig. 6. Switchgrass Harvest. Switchgrass fertilization experiments at harvest time in Tennessee. [Image courtesy T. O. West, Pacific Northwest National Laboratory]

Crop Diversity

Selecting for superior germplasm of any one species in multiple ecoregions will help to minimize inputs, maximize yields, and improve the provision of ecosystem services for that particular species in different parts of the country. Yet it is possible that mixtures of genotypes for a single species or mixtures of species may produce greater or more stable yields in many environments. For example, niche complementarity is a well-known concept in plant community ecology and is a time-tested management strategy in pasture and forage systems. The extent to which such strategies might sustain higher average yields over many years in perennial biofuel systems remains an open question and an opportunity for future research.

Apart from addressing such questions for species that have already been targeted for the development of biofuels, there is scope for discovery of other species, especially locally adapted natives or consortia of native species that could produce equal or greater biomass with fewer inputs (i.e., more sustainably). Perhaps obviously, solutions for sustainable biofuel production will require different species or species mixes in different parts of the country, each optimized for local conditions via breeding.

As novel species and genotypes are developed for biofuel production, evaluating their potential for deleterious impacts on native species and ecosystems will be important. One long-standing concern is that traits enabling high productivity in a plantation setting may also enhance invasiveness in native environments (Raghu et al. 2006). This invasiveness could be exacerbated for germplasm with enhanced stress tolerance and resource use efficiency, especially if those capabilities exceed those of the native flora (Warwick, Beckie, and Hall 2009). For example, one of the main threats to riparian *Populus* populations in the intermountain West is competition from stress-tolerant saltcedar (*Tamarix* spp.) and Russian olive (*Elaeagnus angustifolia*; Nagler et al. 2011). Genetically engineered (GE) crops have been singled out for special concern because of their potential ecological novelty (Chapman and Burke 2006), though some have questioned whether GE crops pose more risk than conventionally bred varieties or exotic organisms (Strauss 2003).

Crops developed for biofuel production are often relatively undomesticated compared to conventional crops because they have been introduced into widespread cultivation only recently or have long generation times and other characteristics that inhibit breeding programs, as is the case for perennial grasses like switchgrass and *Miscanthus* and most woody crops. These undomesticated crops thus have greater potential to survive and thrive outside of plantations compared to conventional crops (Kuparinen and Schurr 2007). These crops often are closely related to populations of wild relatives that grow in the direct vicinity of bioenergy plantations, therefore intensifying the potential for gene flow from pollen and competition with native plants by seed and vegetative propagules (Williams 2010; Kwit and Stewart 2012). Quantifying the amount of gene flow and determining the potential invasiveness of improved feedstocks and their hybrids with native populations will be important (Ellstrand and Schierenbeck 2000). This endeavor will be challenging because of issues related to scaling from small-scale field trials to large-scale releases and the long time frames that must be considered in assessing risks of invasiveness, which has been notoriously difficult for exotic introductions (Sakai et al. 2001). However, the availability of inexpensive and powerful genotyping techniques has greatly enhanced the ability to track gene flow on a landscape scale (Ashley 2010), and information on the relative competitiveness of introduced, GE, and native genotypes can be assessed in well-designed field trials. This information can be integrated with ecological and remote-sensing data in a spatially explicit simulation modeling framework to perform integrated assessments of gene flow risks from bioenergy plantations (Meirmans, Bousquet, and Isabel 2009; DiFazio et al. 2012). Such research will help ensure that biofuel feedstocks that are especially adapted for growth on marginal lands can be deployed on a landscape scale without unintended negative consequences for native ecosystems.

Research opportunities in this area include the following questions:

- What are the optimal species for maximum sustainable production in various ecoregions of the United States?

- Under what conditions are diverse genotypes of a single species or mixtures of biofuel species more productive, stress resistant, and sustainable than monocultures?
- Which other native or introduced species are viable alternatives to currently targeted biofuel species?
- What is the potential for invasiveness of new biofuel feedstocks? What are the characteristics that confer invasiveness and promote gene flow from cropping systems to natural ecosystems?

In Fig. 7. Plant-Microbe Interactions, below, samples are harvested for plant-microbe interaction studies.

Fig. 7. Plant-Microbe Interactions. Harvesting *Populus trichocarpa* root and rhizosphere samples for plant-microbe interaction studies in Oregon. [Image courtesy C. W. Schadt, Oak Ridge National Laboratory] **(Inset images A** and **B)** Confocal microscope images of bacteria on the surface of poplar roots. [Images courtesy J. L. Morrell-Falvey, Oak Ridge National Laboratory] **(Inset A)** Viable *Pseudomonas* sp. GM17 are stained green (with Syto9), and dead cells are stained red (with propidium iodide). The root surface is visualized by autofluorescence. **(Inset B)** *Pantoea* sp. YR343 expressing GFP (green fluorescent protein); the plant root is detected by autofluorescence in the red channel.

2. The Plant Microbiome

Microorganisms have a dramatic effect on plant biology. While some plant-associated microbes are pathogenic, many are beneficial. One of the best-known examples is symbiotic N-fixing bacteria (e.g., rhizobia) that inhabit the roots of legumes and satisfy the plant's N requirements. Microbes also play other critical roles in plant fitness, including the delivery of P and other nutrients to plant roots by mycorrhizal fungi and the provision of growth-promoting hormones by rhizosphere bacteria. The roles of microbes and microbial communities in providing resistance to pathogen invasion and stimulating the plant immune system can also be important.

Plant-associated microbes constitute the plant microbiome, which includes at least three distinct habitats: inside plant tissues such as roots or stems (colonized by endophytes, mycorrhizal fungi, and nodule-inhabiting symbionts), on leaf surfaces (phyllosphere), or in soil adjacent to roots (rhizosphere). Their functions are closely tied to both plant fitness and local and global elemental cycles, including striking impacts on atmospheric greenhouse gas concentrations. For instance, over half the anthropogenic nitrous oxide (N_2O) being added to Earth's atmosphere is now contributed by microbes in agricultural soils, influenced in part by rhizosphere organisms.

Improved understanding of the integral association between plants and microbes has led to a paradigm-shifting view of plants as metaorganisms, or holobionts—the combination of host plant and its associated microbes and viruses—rather than as isolated entities (see sidebar, The Holobiome-Microbiome Concept, this page). Rarely does plant breeding consider the microbiome a selectable trait (pathogen resistance is a notable exception), yet breeding and managing plants as metaorganisms may benefit both sustainable productivity of bioenergy crops and ecosystem services associated with large-scale bioenergy cropping systems.

Increasing bioenergy crop productivity while also meeting societal demands for sustainable agricultural systems requires understanding the genomic and molecular interactions in feedstock plants' immediate microbiome, as well as the biogeochemical processes mediated by microbial communities in surrounding soils. Critical gaps in our understanding of the plant microbiome that must be filled to achieve these goals are identified in the following sections.

The Holobiome-Microbiome Concept

Selection happens in both plants and microorganisms, so treating plants and their associated microbes as single units of selection is important. Plant-associated microbes may live directly within stem, leaf, or root tissues; on leaf surfaces (phyllosphere); or in the soil immediately surrounding roots (rhizosphere). In many cases, these interactions are symbiotic and promote growth of the plant host. Well-known examples include rhizobia, which help plants meet their nitrogen requirements, and mycorrhizal fungi, which provide a variety of nutrients and (may) improve the abiotic and biotic stress resistance of their host. The full scale of plant-microbe metabolic cross communication is not well characterized but is expected to be significant. In one sense, the holobiont can be considered a meta-organism (Zilber-Rosenberg and Rosenberg 2008). Sufficient understanding of the holobiome requires approaching it from a systems biology perspective—understanding the interacting influences of key organisms, from genes to landscapes.

Species Specificity Between Plants and Microbes

Host specificity between leguminous plants and rhizobia strains has been studied intensively, but links between individual cultivars and their microbiomes are not well established in non–N fixing plants. With the advent of high-throughput sequencing, relating the co-occurrence of feedstock cultivars and their microbiomes is now feasible. Identification of the molecular underpinnings of biotic interactions and community composition in managed environments is a rapidly expanding research area, as data from metagenomics, metatranscriptomics, and metametabolomics (the so-called "omics") continue to proliferate. This research potentially could enhance understanding of the genetic rules governing community composition and development and facilitate selection of optimal plant genotypes for long-term deployment in managed settings, as well as the development of new integrated strategies for managing pests and diseases.

Assuming that persistent and specific plant-microbe associations exist in nonleguminous biofuel feedstocks, the next logical question is: what is their functional role? While currently available omics tools may help answer this question, the need is not only for more sequencing, but also for better annotation, better tools for gene and protein prediction, and better high-throughput means for phenotype screening. However, studies testing for rigorous plant-microbe associations should avoid the correlation-causation trap. Also important is recognizing that functional characterizations of plant-microbe interactions cannot be based solely on genomics: (1) the presence of a gene does not necessarily mean it is active, (2) most genomic measurements are not fully quantitative, and (3) current omics measurements are done at a huge scale relative to the true microbial habitat. Although technically challenging, spatially resolved and fine-scale genomics would be an ideal means to connect causal activities of specific microbial phylotypes to high-performing plant genotypes. Alternatively, this approach could provide a useful means to screen for associated microbes that support a particularly favorable plant trait.

Once functional roles are identified, the next two logical questions are: can they be enhanced or promoted in different plant-environment combinations, and, if so, by what means? It is already known that a microbe is unlikely to succeed when simply inoculated into soil, and this difficulty extends to plant growth-promoting microbes in microbiome habitats such as the rhizosphere. Thus, identifying the plant-soil factors that contribute to establishment, growth, and persistence of a favorable species-specific microbiome is another major research challenge.

Research opportunities in this area include the following questions:

- Are microbiomes of particular biofuel plants consistent and persistent through time and across different soil types and climates?
- How does the composition of the microbiome affect a feedstock host plant's fitness and productivity?
- What factors determine the optimal microbial populations and communities for feedstock productivity within and around a plant?
- To what extent do particular microbiomes alleviate feedstock plant stress? Can microbiomes be manipulated to alleviate different biotic and abiotic stresses?
- To what extent can the plant microbiome affect the expression of different plant traits like root-to-shoot ratios and root elongation patterns?
- To what extent is the microbiome controlled by the plant host?
- How do microbes affect soil health (i.e., the soil's ability to sustain plant growth and other valuable biological processes)? How do effects change during plant development and crop establishment?

Rhizosphere Consortia

Defined as the soil influenced by and within several millimeters of a growing plant root, the rhizosphere is a zone of high microbial biomass and activity. The microbes in this part of the plant microbiome are a subset of the background soil microbial community and are influenced by the combination of root exudates, dead cells, and mucilage (collectively known as rhizodeposits) released from a growing root (Philippot et al. 2013). While rhizosphere microbes can be characterized as having a collective influence, rhizosphere composition tends to vary widely from one plant species to another. Rhizosphere organisms have a significant effect on plant fitness and nutrition and have long been studied for their roles in plant N, P, and micronutrient nutrition; growth promotion; and their potential to ward off pathogens (Mendes, Garbeva, and Raaijmakers 2013). As roots grow and eventually senesce, a succession of rhizosphere microbial communities can occur (e.g., Chaparro, Badri, and Vivanco 2014; DeAngelis et al. 2009). However, the overarching importance of this functional and phylogenetic succession is not well understood.

Beyond promoting plant growth and health, rhizosphere microbes also play a significant yet not fully quantified role in soil biogeochemical cycling. In grasslands, where most surface soil is part of the rhizosphere, the importance of rhizosphere microbes is clear. But even where rhizosphere soil comprises only a small portion of the total soil volume, such as annual cropping systems, this zone can provide 30% to 40% of the total organic C input in soil and is a nexus for microbial transformations of soil C (see Fig. 8. Rhizosphere Consortia, this page). Many rhizosphere populations are from phyla identified as fast-growing bacteria (Proteobacteria and Firmicutes), while other major root-responding taxa are commonly associated with macromolecular decomposition in soil (e.g., Actinobacteria and Verrucomicrobia). In some systems, rhizosphere communities have an increased capability for breaking down complex C and N sources and enhancing organic matter decomposition. This priming effect, which is also affected by nutrient availability, could have either a positive or negative impact on soil C stabilization and ecosystem C balance and is an active area of research (Blagodatskaya and Kuzyakov 2008). In general, impacts of variation in microbial composition on ecosystem function (e.g., soil C stabilization, trace gas production, and N and P mineralization) are significant yet poorly understood (Van der Heijden, Bardgett, and Van Straalen 2008).

Fig. 8. Rhizosphere Consortia. As the roots of *Avena fatua* push through soil to acquire nutrients and water, they also provide carbon to a complex microbial community inhabiting the soil environment adjacent to the plant roots. [Image courtesy E. Nuccio, Lawrence Livermore National Laboratory]

The rhizosphere is also a zone of frequent biotic interactions involving the entire soil food web. However, research investigating interactions between meso- and microfauna and microbes often is neglected despite their likely importance in low-input perennial cropping systems, where the absence of tillage and the buildup of soil organic matter could provide suitable conditions for a robust soil food web. The role of viruses in soils surrounding plant roots is another major knowledge gap. Finally, the role of rhizosphere microbiota in conferring disease resistance also remains an active research area. Many of these areas require a comprehensive understanding of the soil microbiota and their interactions with each other, with the soil environment, and with plants. By focusing on interactions of the entire soil food web, including the mesofauna, and using modeling to simplify the complexity of food web interactions, biocontrol strategies could be harnessed to produce all crops in a more sustainable manner.

To address these knowledge gaps, a systems biology approach to plant-microbe interactions is needed. To bridge from genes to ecosystem function, a suite of complementary analyses such as the following might be useful:

DNA → Transcription → Transcripts →
Translation Potential Rate →
Current Environment → Process Rate

Because the majority of rhizosphere microbes are uncultured, culture-independent approaches such as stable isotope probing and strategic omics studies are needed. Most of the current genomic efforts in this area are largely observations and identification of "who is there." Controlled experiments with high-resolution temporal sampling, or studies where small-scale omics investigations are linked to the whole plant and field scale, would be ideal. Also important are spatially resolved technologies and microscale experiments that can more directly link microbial community structure to function and, potentially, even to soil structure and niche quality (Bailey et al. 2012; Davinic et al. 2012;

Kravchenko et al. 2013). These approaches, especially when used in combination, could enable a new understanding of how soil and rhizosphere microorganisms are functionally organized in specific soil-plant systems.

Compelling research opportunities in characterizing rhizosphere communities include:

- What are the most important soil taxa and their functional relationship to biofuel crop vigor?
- Is manipulation of plant-microbe relationships feasible and valuable? Can the community be effectively managed? What are the effects of altering rhizosphere/endophyte community composition, root abundance, architecture, or exudates? Will rhizosphere microbes respond? Will crop yields be affected?
- How do rhizosphere composition and activity affect the turnover and accumulation of stabilized soil C?
- Are there microbial functions that primarily associate with different root zones or pore/niche types in soil? Does the spatial arrangement of these functions reflect resource distribution and transport in the system? If the spatial arrangement of these functions can be characterized, does that provide new insights into how the plant system exploits and mines the soil for resources?
- How are rhizosphere community composition and behavior affected by different plant genotypes, how do effects vary by environment, and what are the molecular drivers for such variation?
- Can crops be bred, or genotypes targeted, to promote the establishment and persistence of beneficial rhizosphere consortia, including those that alleviate stress?
- What genomics knowledge of soil microbial communities is needed to better predict the response of key biogeochemical processes such as C stabilization, denitrification and N_2O fluxes, methane (CH_4) oxidation, and leaching losses to episodic environmental events such as freeze-thaw cycles, prolonged drought, and rainfall events? How will responses differ by crop management strategies (annual versus perennial, woody versus herbaceous, and low versus high inputs)?

Mycorrhizal Fungi and the Mycorrhizosphere

Most terrestrial plants form a symbiosis with ubiquitous soil fungi that consist of filamentous hyphae extending from within the root into the surrounding soil. There are two main types of mycorrhizae—endomycorrhizae and ectomycorrhizae—characterized by the location of the fungal hyphae with respect to root structure. Hyphae of the more common arbuscular mycorrhizal (AM) fungi penetrate into the root cortex intercellularly and intracellularly, whereas hyphae of ectomycorrhizal (EM) fungi only colonize the cortical spaces between cells. Mycorrhizal associations are found in more than 80% of all known plant families (Smith and Read 2008). The more common AM fungi form symbioses with most grasses and field crops (the Brassicaceae are a notable exception), while EM symbiosis occurs mainly in woody plants, including the candidate biofuel crops poplar and willow, which can host both AM and EM fungi.

Mycorrhizal fungi deliver P, N, and other resources to plant roots in exchange for photosynthate-derived C (Sanders and Tinker 1971; Javot et al. 2007; Hodge and Fitter 2010; see Fig. 9. Symbiotic Mycorrhizal Association, p. 17). In addition, mycorrhizal colonization has been found to increase the host plant's tolerance for stress, both abiotic (e.g., drought, salinity, and heavy metals) and biotic (e.g., root and leaf pathogens) (Newsham, Fitter, and Watkinson 1995; Ruiz-Lozano, Azcon, and Gomez 1995; Cameron et al. 2013). Most research has focused on EM fungi in woody species and AM fungi in annual plants (reviewed in Harrison 2005); indeed, the ubiquitous nature of AM fungi in perennial grasses has been revealed only in the past few decades (Van der Heijden et al. 2006). Consequently, knowledge of how these symbioses function in perennial herbaceous plants such as switchgrass is still very limited (Clark, Zeto, and Zobel 1999; Ghimire, Charlton, and Craven 2009). For example, it is largely unknown how AM fungi might provide plant stress tolerance, promote N and P conservation, or be affected by the plant's retranslocation of C and N to roots prior to senescence.

The importance of interactions between mycorrhizae and the surrounding microbial community (mycosphere

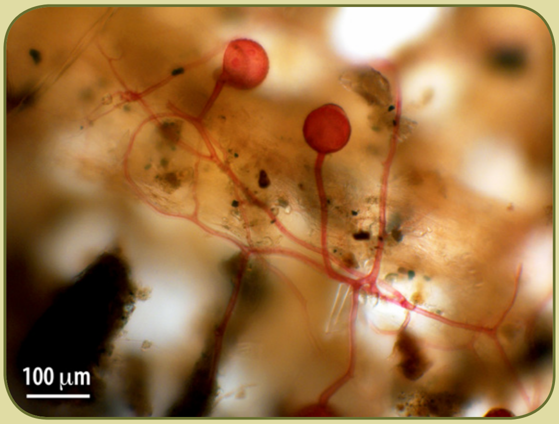

Fig. 9. Symbiotic Mycorrhizal Association. The mycelia of mycorrhizal fungi (*Glomus hoi*) explore decomposing organic matter for phosphorus, nitrogen, and other nutrients to transport to the host plant (*Plantago lanceolata*). Scale bar represents 100 µm. [Image reprinted by permission from John Wiley and Sons Ltd.: Nuccio, E. E., et al. 2013. "An Arbuscular Mycorrhizal Fungus Significantly Modifies the Soil Bacterial Community and Nitrogen Cycling During Litter Decomposition," *Environmental Microbiology* **15**(6), 1870–81. DOI:10.1111/1462-2920.12081.]

as well as endosymbionts) also is poorly understood, although these relationships may be critical to the success of the plant-mycorrhizal symbiosis (Jansa, Bukovska, and Gryndler 2013; Scheublin et al. 2010). These knowledge gaps are related to the fact that there is very little genomics-based information on AM associations. In fact, this area lags seriously behind in the development of genomic platforms. Understanding AM fungi-plant associations and developing the knowledge base necessary to effectively manage and manipulate this ubiquitous association should be given high priority.

To better understand the role of mycorrhizal fungi in seedling establishment, year-to-year persistence, and sustained biomass productivity, genomics technologies should be coupled with functional screens. Such research will help in identifying and characterizing those fungal strains that function optimally with bioenergy crops (and under different environmental conditions) and in understanding the basis for differences in performance across crops and environments. Because AM fungi are obligate symbionts and difficult to grow in culture, developing strategies to screen for beneficial combinations of fungi and plants is an important challenge that needs attention. In addition, bioenergy crops could be screened for natural variation in responses to mycorrhizal fungi, and genomics approaches such as GWAS could be used to identify alleles for a maximum response to fungal symbionts. This information might then be incorporated into bioenergy crop breeding programs.

Compelling research opportunities for characterizing mycorrhizal interactions of bioenergy crops include:

- Among different AM and EM symbioses, what is the basis for differences in function, and can they be utilized to increase feedstock productivity?
- What is the nature of host-symbiont specificity? What are the factors that determine successful inoculation? How do these factors differ by soil characteristics or environmental stress gradients?
- Regarding the genomics of mycorrhizal fungi, which fungal strains are most effective for nutrient uptake and under what conditions, and what makes them effective?
- To what extent do associated microbial communities impact mycorrhizal function (e.g., P and N liberation, capture, and transfer to the plant)? If important, can they be manipulated to enhance function?
- To what extent do mycorrhizae impact the composition and functioning of soil microbial communities and the nutrient transformations that they mediate? How do mycorrhizae influence the identity and function of their associated microbial communities?
- Which mycorrhizal fungal communities are optimal for abiotic (e.g., drought, salinity, and heavy metals) and biotic (plant pathogens) stress resistance of bioenergy crops, how do they function, and how can their effectiveness be increased?
- How do dynamic C fluxes affect the formation and maintenance of mycorrhizal associations in bioenergy plants? How does this ultimately relate to C sequestration in the soil?

Diazotrophs

Nitrogen is the nutrient that most often limits plant growth in both natural and managed ecosystems. Only bacteria and archaea possess the enzyme nitrogenase, which breaks the strong N-N triple bond in atmospheric N_2 and converts N to ammonia (NH_3) in a process called biological N fixation (BNF). It has long been presumed that terrestrial BNF occurs primarily in plants forming symbioses with N_2-fixing bacteria inside root nodules. However, the growing number of non-nodulating N_2-fixing organisms discovered in recent genomic surveys of plant rhizospheres and endospheres suggests an expanding number of means for ecosystem acquisition of fixed N.

Root nodule associations between legumes (Fabaceae) and Alphaproteobacteria such as *Rhizobium* and *Frankia* are well understood and have been studied for decades. Until the advent of molecular phylogenetic studies, many nonrhizobia isolated from nodules were thought to be contaminants and their genomic information (DNA) was routinely discarded (Gyaneshwar et al. 2011). It is now clear that some Betaproteobacteria from the genera *Burkholderia* and *Cupriavidus* are also nodule symbionts.

BNF has been measured outside of root nodules in a wide array of environments, and the emerging availability of nonculture-based techniques to identify microorganisms responsible for N_2 fixation is rapidly revising understanding of this process. Metagenomic analyses of bulk soil (e.g., Wang et al. 2013) point to the common occurrence of *nif* genes, which encode for nitrogenase, in a variety of taxa. Aboveground, endophytic N_2 fixation has been demonstrated on leaf surfaces (phyllosphere; Abril, Torres, and Bucher 2005) and within the stems of sugarcane (Boddey et al. 2003), and N_2-fixing endophytes have been isolated from other biofuel crops including hybrid poplar (Knoth et al. 2014) and *Miscanthus* (Davis et al. 2010). Associations with these diazotrophic microbes could possibly be optimized in feedstock cultivars.

Compelling research opportunities include the following questions:

- How widespread is diazotroph occurrence in biofuel crop rhizospheres, and are they fixing significant quantities of N?
- Is endophytic N fixation important in crops other than sugarcane, and what are the physiological and environmental factors that control its significance? Are inoculants viable? Are they functional singly or in consortia? How can N fixation be maximized for efficient inoculants?
- Can plants be selected that better support associative N fixation either in the rhizosphere or endosphere?
- Can actinorhizal symbionts in *Alnus* and other actinorhizal plants be better optimized for N fixation, thereby increasing their attractiveness as biofuel feedstock species?

Endophytes

Endophytes are nonpathogenic, nonmycorrhizal fungi or bacteria that colonize the interior of healthy plant tissues including roots, leaves, stems, flowers, and seeds (Ryan et al. 2008). They are ubiquitous and can benefit plants by stimulating growth, providing pathogen protection, increasing stress tolerance, and fixing N (see prior section, Diazotrophs). Some endophytes are human enteric pathogens (Tyler and Triplett 2008). More is known about the role of fungal than bacterial endophytes, but endophyte-plant relationships are generally poorly understood. Because many endophytes spend part of their lifecycle outside of plant tissues, reference to these microbes as having an endophytic lifecycle stage may be more accurate. Endophytic microbes also may be recruited directly from the environment. For example, in deserts, free-living bacteria, including members of the Rhizobiaceae as well as certain *Bacillus* spp., are found both in the rhizosphere and within roots (Kaplan et al. 2013). This lifestyle may protect nonspore-forming, gram-negative species from desiccation.

Both model plants (such as *Arabidopsis* and poplar) and wild cultivars are being used to identify endophytes and, importantly, classify their functional roles. Root-associated endophytes such as the dark septate (Mandyam and Jumpponen 2005) and Sebacinales (Weill et al. 2011) fungi appear to be very prevalent throughout the plant kingdom and have been shown to impart various benefits to their host plants. Still, little is known about the *in situ* functional roles of both fungal and bacterial endophytes and whether they could be used to further agronomic goals.

Moreover, little is known about plants' roles in attracting or harboring endophyte populations. Plants recognize their presence and may sense beneficial endophytes in much the same way that they detect pathogens, only with a milder response (presumably involving different receptors) that prevents a full-blown defense reaction. There is also increasing evidence that the environment (e.g., soil), and not necessarily the host species identity, structures the endophyte communities (Schlaeppi et al. 2014). Additionally, many endophytes have a broad host range, which opens up possibilities for isolating endophytes from one plant (e.g., a wild plant growing in N-limited soil) and moving it to another (e.g., a biofuel crop).

Compelling research questions in this area include:

- How prevalent are endophytes in potential biofuel crops, and what is their functional significance to plant vigor?
- Can endophytes be genetically modified or selected to incorporate additional, useful traits into their associated bioenergy host plant?
- What are the mechanisms by which endophytes are recruited from the environment (e.g., rhizosphere), and can these mechanisms be manipulated to increase feedstock productivity?
- What controls the prevalence of human enteric pathogens in plants, and can other endophytes be used to limit their entry?
- Can synergistic, beneficial effects be obtained by combining various plant-microbe symbioses?
- Which conditions disrupt healthy plant-endophyte associations or limit the functional benefits of the symbiosis?

Pathogens and Insect Pests

A wide variety of pathogens and insect pests is known to affect biofuel crops, thus influencing plant population sizes, community composition, and ultimately biomass yields. Insects cause direct damage by removing plant biomass and can indirectly harm plants by vectoring pathogens. Soil-borne pathogens tend to build up in the rhizosphere, and current limited mechanistic understanding of the processes involved with their movement into plants and subsequent plant responses presents important knowledge gaps. Pathogens also are delivered to foliar plant parts and, via aphid and other insect vectors, to vascular tissues. The extent to which plants can differentiate between beneficial microbes and pathogens and act to differentially promote or exclude them is an important determinant of plant success.

In annual crops, breeding for resistance to specific pathogens and insect pests and optimizing residue management and crop rotations have been important strategies for limiting pest success. These strategies have not been extensively studied in perennial biofuel crops, where long lifecycles slow breeding progress and rotations can last decades. Consequently, understanding microbial community dynamics in the rhizosphere, phyllosphere, and endosphere is crucial, as well as learning whether and how microbial assemblages might be managed to deflect pest impacts or increase signaling to pest antagonists.

For example, many naturally occurring rhizosphere bacteria and fungi are recognized as being antagonistic toward crop pathogens. Soil-borne pseudomonads have been used as biocontrol agents in organic agriculture. Mycorrhizal fungi may also play a role in endophyte recognition and exclusion and, by extension, in the exclusion of pathogens.

Key research questions surrounding pathogens and insect pests include:

- How do plants differentiate between pathogens and mutualists?
- To what extent might plants influence their microbiome to be pest resistant? Can plants be bred to favor microbiomes antagonistic to pathogens or to be capable of signaling a pest's natural enemies?
- How might microbial communities in the rhizosphere, phyllosphere, and endosphere be managed to confer pathogen resistance?

Synthetic Biology

Synthetic biology focuses on *de novo* engineering of genetic circuits and the biological processes they encode and control. Targets of synthetic biology may include plant feedstock species as well as the microbial species that affect them. A challenge on the microbial side will be to ensure the competitiveness and persistence of novel, introduced microbes in the rhizosphere and other parts of the plant microbiome. One approach is to start with competitive microbial species and strains that already are good colonizers of root surfaces or root tissues and endow them with additional functions. This approach may be simpler than starting with microbes that have desirable functions and endowing them with "competitiveness" genes. For instance, *Pseudomonas fluorescence* Pf5 (a non–N fixing root epiphyte) and *Rhizobium* sp. IRBG74 (an N-fixing root endophyte) are currently the focus of efforts to engineer synthetic N-fixing symbioses between these microbes and the model C4 grass, *Setaria viridis*, or its crop relative, *Zea mays* (corn). Alternative approaches to providing plants with N via BNF include engineering legume symbiosis into nonlegumes and engineering expression of nitrogenase into plants rather than bacteria (Oldroyd and Dixon 2014).

Important research questions in this area include:

- Which novel functions or constellations of functions can be introduced into plants or microbes to enhance the resilience and yield of biofuel crops under low-input (e.g., water and nutrients) and otherwise challenging environmental conditions?
- Can biofuel plant species or their symbionts be engineered to fix atmospheric N_2 and reduce the need for industrial N fertilizer and losses of reactive N to the environment?
- Can synthetic biology approaches be deployed in plants or microbes to reduce biogenesis of the greenhouse gases CO_2, CH_4, or N_2O?
- How might beneficial microbes be engineered to make them better able to survive and thrive in existing microbial communities?
- What is the role of plant exudates in promoting a more beneficial rhizosphere community, and can plant systems biology be used to make rhizospheres better habitats for beneficial microbes?
- How ubiquitous are "lock and key" relationships, whereby the plant provides a key exudate for a specific beneficial microbe?

3. Ecosystem Processes

Environmental sustainability is largely expressed at the ecosystem and larger scales of landscapes and regions. Biofuel ecosystems (individual biofuel crop fields) capture and sequester C, mitigate greenhouse gas fluxes, regulate water and nutrient flows to aquatic systems and other parts of the landscape, and provide habitat for organisms that benefit both crop and natural communities. These organisms include pollinators, biocontrol agents such as natural enemies of crop pests, and birds of conservation value. All these attributes and processes will be affected by the establishment of biofuel crops on lands that now host ecosystems with different plant communities managed at different levels of intensity.

Conversion to biofuel cropland will thus result in the delivery of a set of ecosystem services different from that before conversion. The net contribution of biofuel croplands to environmental sustainability depends on many interacting factors, almost all of which are influenced by how the crop and its associated microbiome interact. Because these interactions will differ by crop, location, soil type, and management practices, a sufficient fundamental understanding of their ecosystem-scale effects is needed to enable predictions of aggregate effects at landscape and regional scales. Such understanding will help to gauge net benefits and avoid unintended environmental consequences.

Carbon Capture and Sequestration

The processes of C capture by plants and its fixation into plant biomass, turnover and deposition in dead plant material, and return to the atmosphere as CO_2 or CH_4 by decomposers are the key dynamic fluxes of the terrestrial C cycle. The quantity and residence time of C in living or dead biomass and in soil organic matter pools generally define the amount of C captured and stored by terrestrial ecosystems.

Conversion of existing lands to bioenergy cropping systems will alter ecosystem C fluxes, as well as the size and residence time of stored C pools, with ramifications for greenhouse gas concentrations in the atmosphere. In bioenergy cropping systems, the harvest and removal of most aboveground plant biomass restrict long-term ecosystem C capture largely to belowground pools. Thus, the amount of belowground C inputs, stability and residence time of soil C pools, and soil's native ability to store C (West and Six 2007) are key factors that will determine the capacity and duration of C sequestration afforded by bioenergy cropping systems.

Soil C storage is a major determinant of a biofuel cropping system's greenhouse gas balance and thus of its climate mitigation potential. Soil C is typically lost via microbial oxidation during the establishment phase of any biofuel cropping system, which is every year for annual crops and 1 to 2 years following planting for perennial crops (Gelfand et al. 2011). Minimizing establishment phase C loss and maximizing soil C recovery represent a crucial strategy for repaying biofuel C debt. In systems where much soil C is lost or cannot be repaid quickly (e.g., maize plowed annually), the net ecosystem greenhouse gas balance can be climate negative for more than a century, even including the fossil fuel offset credit from converting biomass to fuel. Conversely, in systems where little soil C is lost during establishment or can be repaid quickly (e.g., no-till switchgrass), the net ecosystem greenhouse gas balance can be climate positive within 2 to 3 years.

In addition to its climate mitigation benefit, the C captured and stored by bioenergy crops can contribute substantially to soil fertility—another ecosystem service and one that will in turn benefit future biofuel crop productivity. Soil C is stored as soil organic matter, which serves as a valuable nutrient reserve, helps regulate nutrient cycling, improves soil structure, and increases infiltration and water-holding capacity, all of which contribute to sustainable plant production systems. Additionally, soil organic matter helps reduce erosion, runoff, and flooding; mitigate drought; and provide clean water by filtering and degrading contaminants.

Predicting the impact of different bioenergy cropping systems and their associated microbiomes on belowground C capture and stabilization in living biomass and soil organic matter pools requires an improved understanding of complex plant-soil-environment interactions at multiple scales. Alterations in plant biomass production, biomass allocation between above- and belowground structures, the lifecycle of these structures, and substrates released during plant growth interact with the soil microbial community, other decomposers, and the soil's physical and chemical environment. These reactions control the amount of C captured below ground, the location of that C, and its long-term stability (von Lützow et al. 2006; Jastrow, Amonette, and Bailey 2007; King 2011; Stockmann et al. 2013). In particular, research is needed to better understand the mechanisms and processes controlling the types and rates of C inputs to and outputs from the belowground systems of bioenergy crops. This research also must be sufficiently robust to account for variable effects associated with different bioenergy crops, soil types, edaphic conditions, management practices, and climatic regions.

The establishment of different bioenergy crops (in monocultures or polycultures) and the selection or breeding for different plant traits can lead to a variety of intended or unintended impacts on belowground C inputs and subsequent loss or sequestration. Furthermore, these impacts must be understood in the context of the tradeoffs and consequences of optimizing for feedstock production, sustainability, and C sequestration and be evaluated on a full C-cost (greenhouse gas) accounting basis (Ravindranath et al. 2009; Robertson et al. 2011).

Plant biomass production and its allocation to above- and belowground structures are primary factors affecting belowground C inputs. The amount of belowground biomass production varies with species and cultivars. However, within species, breeding and selection efforts to increase aboveground yield may reduce belowground production and thus lower the rate of C inputs to soil, while selection for greater root-to-shoot ratios might enhance belowground C storage at the expense of yield. Similarly, the choice of annual versus perennial crops affects biomass allocation and C inputs. Annuals often invest less energy in belowground structures compared to perennials, but at the end of the growing season the entire root system of an annual crop becomes a source of belowground litter, whereas only a portion of perennial root systems turns over each year. Thus, the effects of bioenergy crop production and biomass allocation on C sequestration depend heavily on crop structure turnover times.

More subtle variations in plant traits also have the potential to affect C inputs and sequestration. Crop selection or breeding that alters traits such as the spatial distributions of roots, their morphology and anatomy, root tissue chemistry, exudation rates, and associations with mycorrhizal fungi can affect water and nutrient uptake, decomposition, and C sequestration. Traits that allow for greater root growth at deeper depths might increase sequestration by placing more C where the physical and chemical soil environment might be less conducive to rapid decomposition. Root morphology and anatomy, tissue chemistry, and exudates influence the chemical and structural composition of C inputs, which also can affect the composition, biomass, and activity of the rhizosphere microbial community, including the associative N fixers. Such alterations in plant-microbe interactions could affect the rate of C and nutrient cycling in the rhizosphere and might also prime or retard the mineralization of existing soil organic matter pools, all of which could affect C sequestration.

Further, changes precipitated in the composition, size, and turnover of the microbial community have implications for the quantity and nature of C inputs to soil organic matter derived from microbial residues. Similarly, allocation of plant photosynthate to support mycorrhizal fungi significantly alters the physical size, chemistry, spatial distributions, and turnover times of C inputs (compared to those derived from plants) and, therefore, their potential for stabilization in soil. All these interactions of living components and residue inputs will ultimately influence C sequestration through effects on soil food webs, such as potential changes in trophic level composition and interactions that could alter grazing, predation, and decomposition rates.

Edaphic properties also play a role in C cycling and sequestration. In a manner similar to their impact on plant growth, edaphic properties affect the overall habitat of and resource availability for soil microbes and other members of the soil food web. The stability of existing soil C and capture of new C inputs are dependent on the interactions of organic materials with the soil matrix. Thus, variations in soil characteristics (e.g., particle-size distribution, type and reactivity of clay minerals, quantity of exchangeable cations, pH, redox conditions, and soil structure) can exert strong controls on the amount and stability of C captured and sequestered under bioenergy cropping systems.

The complexity and importance of soil C changes in biofuel cropping systems raise a number of compelling questions that include:

- Which bioenergy crops or cultivars provide the best balance between feedstock production and C sequestration? Can plant breeding or selection efforts improve this balance?
- Which feedstock plant traits can be manipulated through breeding and selection programs to enhance soil C sequestration via changes to the quantity, quality, and location of belowground C inputs?
- Can the function and activities of the microbial community, including mycorrhizal fungi, be manipulated via alterations of biofuel plant traits or other means to optimize C sequestration?
- What metagenomic knowledge of soil microbial communities is needed to enable better predictions of C sequestration or depletion?
- How do different edaphic conditions affect the rate of C accumulation under different bioenergy crops and ultimately its potential stability and residence time?

Greenhouse Gas Mitigation and Albedo Change

All three major biogenic greenhouse gases—CO_2, N_2O, and CH_4—are affected by land use and agronomic management. The CO_2 captured in biomass that is then converted to liquid transportation fuel can offset significant amounts of fossil fuel and, as noted previously, the CO_2 converted to stabilized soil C can effectively sequester CO_2 from the atmosphere. Alternatively, crop and management decisions can substantially diminish the fossil fuel offset, and some management practices will result in soil C loss rather than gain. Anthropogenic fluxes of N_2O and CH_4 are dominated by agricultural sources both globally and in the United States, and growing atmospheric concentrations can be either mitigated or exacerbated by new biofuel cropping systems. Again, discerning and managing the direction and magnitude of change require a fundamental understanding of the microbial sources of these gases and the factors that regulate the capacity of microbes to produce greenhouse gases, including genomic and environmental factors within and outside plant influence, most directly expressed via the rhizosphere. Additionally, the choice and management of biofuel crops will affect land surface reflectivity—albedo—which is an important additional influence on global temperature change (see Fig. 10. Flux Tower Measurements, this page).

Fig. 10. Flux Tower Measurements. Researcher checks instrumentation on a carbon dioxide flux tower in a switchgrass field in Michigan. [Image courtesy B. Zenone, Michigan State University] **(Inset A)** Radiometers mounted on an eddy flux tower above a switchgrass field in Tennessee. **(Inset B)** Sonic anemometer and gas analyzer mounted on an eddy covariance tower measuring fluxes of energy, water, and carbon dioxide in a switchgrass field in Tennessee. [Inset images A and B courtesy T. Martin, Argonne National Laboratory]

After CO_2, N_2O is the most important greenhouse gas in all major cropping systems except lowland rice, including biofuel crops. Two groups of microbes produce N_2O: denitrifiers and nitrifiers. Denitrifiers are heterotrophs that produce N_2O by respiring nitrate instead of oxygen (O_2) in microhabitats where O_2 is low or absent. In cropped soils, this process typically occurs inside soil aggregates where O_2 can be consumed faster than it can diffuse into the aggregate from the bulk soil atmosphere. During denitrification, nitrate is transformed to N_2O, some or all of which can escape to the atmosphere prior to its further reduction to N_2 gas. Nitrous oxide is also formed during nitrification, the autotrophic oxidation of ammonium to nitrate. Neither the mechanisms that control the proportion of denitrifier endproduct that becomes N_2O nor the conditions and underlying mechanisms that lead to nitrifier N_2O production are fully understood.

The N_2O produced from either microbial source is largely controlled by soil N availability. In both cropped and unmanaged ecosystems, the single best predictor of N_2O flux is the availability of inorganic N, whether supplied by synthetic fertilizer, organic fertilizer, or BNF. This explains, in part, why fertilized agricultural systems are the major source of anthropogenic N_2O globally. Also, because atmospheric N_2O has ~300 times the global warming potential of CO_2, N_2O typically dominates the overall greenhouse gas balance of annual cropping systems.

Unfertilized perennial biofuel cropping systems produce very little N_2O, typically no more than unmanaged successional ecosystems at the same geographic location. Nitrogen fertilization increases N_2O fluxes in the same manner as for annual crops, and accumulating evidence suggests that fluxes increase exponentially with added N that exceeds plant needs. Thus, N fertilizer management and crop N acquisition can have a huge impact on the climate mitigation potential of cellulosic cropping systems.

In most upland soils, CH_4 is consumed by bacteria that oxidize CH_4 to CO_2 at rates that exceed CH_4 production, so soils that are not submerged are a net sink for atmospheric CH_4. Globally, CH_4 consumption is of the same order as CH_4 accumulation in the atmosphere, and thus CH_4 oxidizers (i.e., methanotrophs) play an important role in the global CH_4 cycle. However, CH_4 oxidation is suppressed by agriculture (particularly by N fertilization) to rates that are <30% of those in uncultivated ecosystems (Del Grosso et al. 2000). Methane oxidation recovers during ecological succession, suggesting that the capacity also might be restorable in perennial biofuel cropping systems, so long as available soil N stays low. Why CH_4 oxidation recovers so slowly during succession is unclear. Circumstantial evidence suggests that microbial community composition may play a major role, and edaphic factors are likely to be important as well. If methanotrophs are part of the plant microbiome, then plant breeding might be used to promote the recovery of CH_4 oxidation in biofuel cropping systems.

Albedo refers to solar reflectance, and changes to the albedo of cropped ecosystems can have a significant effect on the system's energy balance. Plant canopies with high reflectance absorb less light and re-emit less heat, leading to cooler surface temperatures. The net difference in albedo between a cropping system and the natural vegetation it replaced affects the system's climate impact. Albedo usually is not considered in biofuel lifecycle analyses, although perennial biofuel cropping systems are likely to have very different albedos from annual cropland and perhaps from natural communities where aboveground biomass is preserved during the winter.

Compelling research questions regarding greenhouse gas mitigation and albedo change that deserve further exploration include:

- How do candidate biofuel cropping systems differ in their propensities to produce N_2O and in the relative amounts of N_2O from denitrifiers versus nitrifiers? To what extent do other taxa, including fungi, contribute to N_2O fluxes? Does the plant microbiome play a role?
- What is the basis for differences among denitrifiers' abilities to reduce nitrate all the way to N_2 versus stopping at N_2O? Can plants influence the N_2O:N_2 ratio? How does microbial community composition affect this ratio?
- How do candidate biofuel cropping systems differ with respect to CH_4 consumption? Do soil microsites differ in their consumption potential? Can management practices be used to alter microbial

communities in ways that improve CH_4 consumption? What role do plants play in the biofuel cropping system's capacity to consume CH_4?

- How do changes in surface albedo affect the climate mitigation capacity of biofuel cropping systems? Do candidate biofuel species and the way they are managed significantly affect albedo? If important, can breeding and management be tuned to optimize albedo?

Hydrology, Water Quality, and Biodiversity Services

Crop type and management can have a substantial effect on evapotranspiration and, consequently, the water balance of a given agricultural landscape. Land use change that substitutes one type of plant community for another, such as a biofuel community, will create hydrologic change. First, in the absence of differences in WUE (see section, Resource Use Efficiency, p. 6), more productive plant communities will evapotranspire more water, leaving less for runoff and percolation to groundwater and then surface waters including, for example, streams, rivers, lakes, and wetlands. In extreme cases, greater evapotranspiration can lead to the disappearance of intermittent streams and small wetlands. Second, changes in phenology can affect the portion of the year when evapotranspiration occurs; annual cropping systems typically evapotranspire for only a few months in contrast to perennial systems that may evapotranspire for most of the year, with consequent effects on temporal water flow patterns in cropped landscapes. Third, changes in vegetative cover and edaphic properties, such as compaction and soil organic matter content, will affect the proportion of the remaining water that runs off in overland flows versus the amount that infiltrates to groundwater.

The quality of water that leaves an agricultural landscape is strongly affected by its hydrology. Surface runoff, for example, promotes the movement of soil particles into surface waters, bringing with it clay, silt, sand, dissolved organic C, and adsorbed elements such as particulate C, N, and P. The result is turbidity, sedimentation, and eutrophication. Infiltration, on the other hand, leads to nutrient leaching into groundwater where excess soil nutrients are available to be leached. Nitrate, for example, is readily transported to groundwater and eventually coastal regions where it can create offshore hypoxic zones. Recent research suggests that as for N_2O loss (above ground), nitrate loss increases exponentially in fertilized biofuel systems once plant N demands are met.

Water demands are likely to vary significantly among biofuel crops. WUE differs widely between C3 and C4 species. For example, C4 crops can have twice the WUE of C3 crops in otherwise similar and warm settings. Even within these functional groups, WUE can vary markedly with different life history traits such as growth rates and phenologies, root-to-shoot ratios, and root architectures. Thus, choice of crop (and cultivar) and where it is grown in the landscape will have strong consequences for a cropping system's water footprint, a concept used to describe the combined considerations of WUE, water demand, and water quality impacts.

Agricultural landscapes support plant, animal, and microbial communities that provide a variety of biodiversity services. Informed choices regarding biofuel crops and their placement within the landscape can increase a number of these services simultaneously, including pest suppression, pollination, CH_4 oxidation, and conservation of threatened and endangered bird species (e.g., Werling et al. 2014). Understanding how bioenergy landscapes can be designed to optimize multiple services remains a significant challenge.

Important outstanding issues with hydrologic, water quality, and biodiversity services center on the general question of how biofuel cropping systems can be designed to optimize multiple services. More specific research questions include:

- How can WUE at the ecosystem scale be maximized by crop selection and breeding, and what, if any, are the yield tradeoffs?
- What are the key factors that minimize nitrate, P, and erosion losses, and to what extent can plants be genetically altered or bred to promote nutrient and soil conservation through changes in composition, rooting patterns, and architectures?
- How can N fertilizer levels be optimized to maximize productivity and minimize leaching losses?

Does N fixed from biological sources behave differently from N provided by fertilizers with respect to leaching losses?

- At landscape scales, how will widespread implementation of biofuel crops influence water quality, pest suppression, pollination, and biodiversity services?

4. Multiscale Modeling

The biological processes underpinning sustainable biofuel production systems are inherently complex and have important emergent properties across spatial and temporal scales, from molecular to landscape and from minutes to decades. Multiscale modeling is an irreplaceable tool for understanding the behavior of complex biological systems. Such modeling integrates mechanistic models describing system performance at discrete biological scales to evaluate the behavior of whole systems. Multiscale models enable the evaluation of system behavior in a range of spatial (e.g., from soil pores, to fields, to watersheds or regions) and temporal (e.g., from hourly and daily processes to decadal consequences) contexts, including future climate and management scenarios. Such models are most useful if they explicitly simulate biological mechanisms to achieve model results that accurately extrapolate system behavior beyond the specific empirical cases used to obtain system parameters (Nair et al. 2012). An important value of mechanistic models is that they can reveal knowledge gaps that require additional empirical research. Another important value of mechanistic models is their ability to identify key processes through sensitivity analysis (see Fig. 11. SimRoot Simulation, this page).

Opportunities to develop multiscale mechanistic models are expanding because of the growing availability of large datasets at the genomic and ecosystem scales and the increasing accessibility and power of computational resources. To develop meaningful multiscale models that can integrate system behavior from genomes to landscapes and from the subsurface to the troposphere, more mechanistic information is needed about the biology of plant tissues, organs, and whole plants and how they interact with the abiotic environment and other organisms. Furthermore, more information is needed about microbial cells, populations, and communities and their interactions with plants. These needs are especially true for the root-rhizosphere phenome, which is (1) poorly understood; (2) served by few cross-disciplinary research teams with expertise in plants, microbes, and soils; and (3) key to plant-soil interactions, resource use efficiency, and thus the sustainability of biofuel agroecosystems. An additional constraint to research in the United States is very weak training and research capacities for mechanistic modeling at supramolecular scales. Further, existing modeling efforts are not well integrated, including the linkage of downscaled climate models to quantitative crop, biogeochemical, and other biotic models.

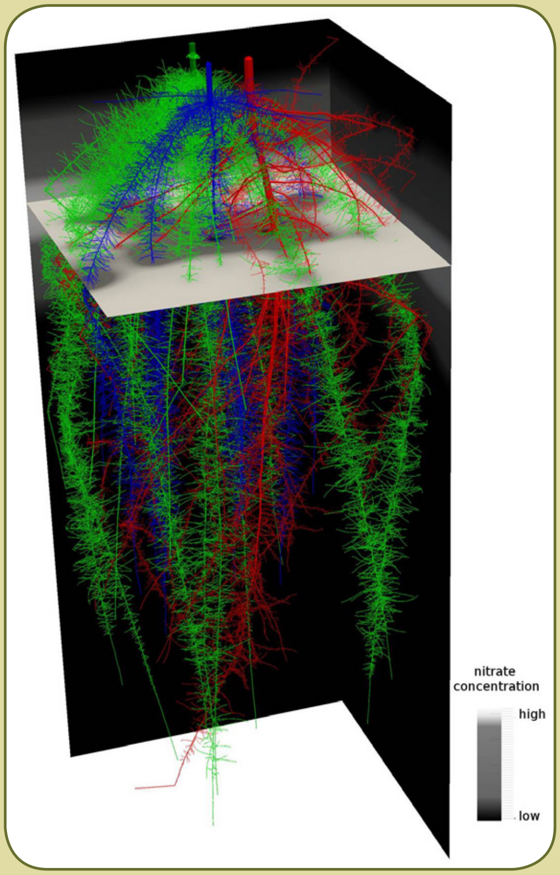

Fig. 11. SimRoot Simulation. A functional-structural plant model, SimRoot, was used to visualize the root architectures of maize, bean, and squash as they competed for a pulse of nitrate in a leaching front. [For more details, see Postma and Lynch 2012.]

The application of mechanistic and multiscale models would have value for all the other research opportunities discussed in this report. Indeed, modeling is most valuable when integrated with empirical research by multidisciplinary teams generating new knowledge, so that modeling and empirical research can inform each other in an iterative manner. In addition to this distributed modeling effort, the community would benefit from the development of centralized modeling resources including software tools, modules, and open-source standards in addition to the spatial databases needed to extrapolate across large regions.

Important outstanding research questions concerning the role that multiscale models can play in the design and evaluation of sustainable biofuel cropping systems include:

- What are the emergent genomic properties of biofuel cropping systems that potentially could transform the predictive capabilities of process-based models?
- What are the possibilities for developing and testing a multiscale modeling system that is capable of accounting for key processes ranging from gene networks to ecosystems, watersheds, and regions?
- How can such a multiscale modeling system help evaluate biorefinery-scale and regional-scale biofuel production systems?
- How can multiscale modeling systems incorporate biodiversity processes to achieve a better understanding of the impacts biofuel cropping systems have on landscape-scale biodiversity services?

Research Infrastructure Needs

The research needs identified in this report will require a new approach and new tools. Major needs include (1) field sites where long-term systems-level research can be conducted, (2) new instrumentation to link advances in the genomic and ecosystem sciences and take full advantage of new technological developments, (3) computational and information management resources to provide necessary visualization and modeling capacities, and (4) workforce support to train the next generation of biofuel systems scientists.

1. Field Sites

Common to all the previously described research questions are two underlying assumptions. First, because sustainability is the net sum of many different attributes and processes and because most, if not all, processes are interrelated, the outcome of one process affects others, so questions must be addressed in a systems context (see Fig. 4, p. 8). While initial discoveries may result from observations and experimentation in narrowly defined experimental settings (often in the laboratory or greenhouse), their importance and relevance to sustainable bioenergy production must be explored in the context of entire cropping systems where key interactions can be evaluated *in situ* along the entire continuum of genes-to-ecosystems and then extended to landscapes and regions via modeling.

Second is the need to evaluate systems over relevant temporal scales. Many ecological processes whether in natural or agricultural ecosystems occur over long time periods. Soil carbon (C), for example, can take many decades to reach a new steady state following land use or management change. Soil microbial communities (e.g., methanotrophs) likewise can take decades to reorganize following changes to the plant-soil environment, and all ecological processes are subject to natural environmental variability that may be increasing with changing climates. Some variability is climate related and thus is subject to long-term changes. In some parts of the United States, growing seasons are lengthening and periodic droughts and wetter springs are becoming more frequent, as are episodic events like heavy storms and heat waves. Biotic change is equally variable and important. Insects and pathogens also respond to climate change, and pest outbreaks occur unpredictably.

Because perennial biofuel systems are expected to persist for a decade or longer after an establishment period of several years, evaluating the performance of key processes and interactions becomes especially important over multiyear, even multidecade, periods. Even short-term questions need to be evaluated for periods that encompass a reasonable level of environmental variability. Moreover, breeding cycles for perennial plants also demand long time periods of study. The net result is a compelling need for field sites where both long- and short-term questions can be pursued in a systems context.

The spatial component to variability raises an additional need to locate field sites in different climate-soil locations. A reasonable approach would be to create a network of sites in broad ecoregional zones such as subsets of the 28 Land Resource Regions under the U.S. Department of Agriculture's (USDA) Natural Resources Conservation Service, 20 ecoregions under the National Science Foundation's (NSF) National Ecological Observatory Network (NEON), or 9 farm resource regions of USDA's Economic Research Service. Modeling would be used to extend results to other locations, supported by smaller test sites on different soils in each ecoregion.

2. Instrumentation Opportunities and Needs

Biofuel sustainability research will profit immeasurably from the use of existing Department of Energy (DOE) user facilities. The sequencing capacity of the DOE Joint Genome Institute, for example, can provide high-throughput genome sequencing, crucial for addressing both plant and microbial sustainability research questions. The ability of DOE's Environmental Molecular Sciences Laboratory to provide noninvasive visualizations of contrasting plant morphologies via whole-plant magnetic resonance imaging and high-resolution and spatially resolved analyses of C chemistry offers an unprecedented means to quickly evaluate phenotypic variation in vascular and other plant tissues. Lawrence Berkeley National Laboratory's Advanced Light Source also has a range of capabilities for spatially resolved analysis, including (1) synchrotron-based Fourier transform infrared microspectroscopy that can be used to investigate delicate living structures without damage, (2) microtomography that enables three-dimensional (3D) imaging of soil and plant structures, and (3) multiple soft X-ray techniques capable of providing detailed chemical information on complex organic structures. Argonne National Laboratory's Advanced Photon Source has new imaging technologies that can provide 3D microtomographic visualizations of soil aggregates and rhizospheres (crucial habitats for the microbiome) and resolve C chemical speciation in samples without the need for cryopreservation or high vacuum.

Advanced instrumentation for field use is also needed. Rhizotrons that enable examination of *in situ* root growth, architecture, and turnover can, especially when coupled with automated image analysis, provide invaluable insights into interactions between plant roots and soil and the allocation of C and other plant resources to root structure and function. *In situ* root exudate sensors and sippers can provide information about relationships between roots and the rhizosphere inhabitants that metabolize the myriad C compounds that roots release. Likewise, new microdialysis methods (Inselsbacher et al. 2011) can be used to observe inorganic and organic nitrogen (N) compounds in soil, and fluorescent substrates and sensors can be used to visualize the activity of various enzymes in soil at micrometer scales.

In situ isotopic imaging can provide quantitative measures of the dynamics of C, N, and other nutrients essential for plant and microbial growth, providing insights into nutrient cycling at the scale of individual soil particles and plant tissues. Such measurements are valuable, for example, to quantify N_2 fixation *in situ*. Isotopic field analyses, including isotopomer analysis, also would be invaluable for real-time measures of nitrous oxide sources in different biofuel cropping systems. Short-lived radioistopes (e.g., ^{11}C) can be used as metabolic tracers for real-time, nondestructive imaging of plant metabolism and soil-plant interactions via positron emission tomography (PET) or single photon emission computed tomography (SPECT). Optodes and similar fiber optic sensors are now available for *in situ* sensing of oxygen, carbon dioxide, and pH and could be developed for nitrate and other ions, providing further insights into microhabitats and soil nutrient dynamics. Automated gas flux chambers are needed to quantify short-term gas fluxes from soil at temporal scales sufficient to capture plant influences that change diurnally.

A third set of field instrumentation needs includes coordinated phenotyping platforms that would permit the in-depth standardized analysis of an array of phenes within specific genotypes across multiple environments. From 30 to 5,000 genotypes might be phenotyped for multiple traits at many different locations; for example, a standard reference set of different switchgrass varieties representing an array of sustainability traits might be planted and evaluated in multiple locations. Combined genetic and phenotypic analyses of these reference sets would enable meta-analytic approaches not otherwise feasible. Notably, evaluation of the same set of genetic lines in various environments permits analysis of the genetic basis of genotype by environment (G×E) interactions, as well as analysis of the physiological basis of environmental responses and adaptations that are essential for understanding system sustainability. Coordinated, standardized phenotyping platforms would also facilitate the engagement of researchers with a range of disciplinary foci and research capabilities in common projects that foster new insights and perspectives.

3. Cyberinfrastructure Needs

Cyberinfrastructure describes the computational instrumentation and software needed to advance sustainable biofuel systems. Computational resources are needed for three broad tasks. First is the need to organize and share data across multiple field sites, experimental systems, and disciplines. Because systems research generates data heterogeneous in scope and scale, the need for investigators working in one part of the system to have access to and understand information generated in another is crucial to successful integration. Often these needs are *post hoc* as discoveries uncover relationships not previously apparent, so the data need to be managed in a manner that is independent of individual researchers and with appropriate metadata and provenance records. Though genomic data have been managed in such a manner for many years, this is not the case for ecological data other than for programs such as NSF's Long-Term Ecological Research Network and its NEON program. Thus, substantial effort would need to be directed toward meeting information management needs, building on or closely integrated with the DOE Systems Biology Knowledgebase.

A second need is the development of software tools for phenotypic data. Phenotyping benefits from a common set of traits or phenes (see sidebar, Phenotyping Plants, p. 7) being measured, with a common set of measurement standards or protocols and data shared in community databases. These standards need to be defined. Phenotypic data are inherently more complex than genotypic data and rely on metadata such as environmental conditions for correct interpretation. Environmental data, including management, soil, and weather conditions, need to be linked to phenotypic data, ideally using standard metrics that permit integration across years, sites, and research teams. Software tools to search, cross-reference, and integrate across these phenotypic datasets with their associated metadata are also needed.

Finally, computational resources are needed for running high-resolution models and to improve standardized databases important for high-resolution spatiotemporal modeling across large scales. Many of these resources currently are available at multiple DOE laboratories, though additional capacity might be necessary. However, standardized databases for soil, land cover, and land use are not uniformly available or validated across regions appropriate for biofuel cropping systems. Effort is needed to improve data coverage and validity using remote-sensing and other tools.

4. Workforce Training

The availability of U.S. expertise in several disciplines important to biofuel sustainability (as outlined in this report) is diminishing. In other disciplines, this expertise needs bolstering to foster the interdisciplinary approaches required to address questions in a systems context. Disciplines that need additional support include plant ecophysiology, mechanistic multiscale modeling, and organismic biology. Few researchers and research teams have the breadth of knowledge in plant biology, microbiology, soil science, ecology, and modeling to address the challenges posed in this report. Critical gaps exist between soil science and plant biology, and between genome and phenome biologists. Although DOE does not have a mandate for training the U.S. scientific workforce, it can encourage interdisciplinary/cross-curricular education by prioritizing integrative projects and supporting interdisciplinary team efforts that take a comprehensive systems biology approach.

Most U.S. graduate and postdoctoral training requires students to acquire deep expertise in one subject area. However, to address pressing environmental questions, a workforce trained to work collaboratively across research areas is needed. For example, training is required to ensure sufficient breadth to enable knowledgeable collaboration among genomicists, system modelers, plant ecologists, soil scientists, and ecosystem scientists.

Compendium:

Challenges and Opportunities for Linking Genomic and Ecosystem Sciences in the Development of Sustainable Biofuel Systems

Recent advances in "omics" and systems biology technologies together with increasing computational resources are enabling the investigation of mechanisms underpinning plant-microbe-environment interactions at a level never before possible. Using these tools to link the genomic and ecosystem sciences will bring new understanding of how the environment influences these interactions, how these interactions affect ecosystems, and how computational power can be used to accurately predict key outcomes for different plants in different ecosystems within a changing climate. The goals of the Research for Sustainable Bioenergy Workshop were to assess current understanding of (1) the influence of biotic, abiotic, and genetic variables on long-term plant feedstock performance and (2) the delivery of potential ecosystem services at multiple scales. Ultimately, building on this understanding will lead to novel ways to link genomes and ecosystems using the tools of systems biology, genomics, and ecosystem science. Such insights will prove invaluable in facilitating the development of bioenergy feedstocks that are highly productive and sustainable at multiple scales.

Research opportunities were identified in four separate but integrated categories:

- ***Plant Systems*** includes opportunities related to plant productivity, nutrient and water use efficiency, genotype/phenotype breeding, and crop diversity.
- ***The Plant Microbiome*** includes opportunities related to microbe-plant species specificity, particularly rhizosphere microbes and their plant interactions, mycorrhizal fungi, diazotrophs, endophytes, pathogens and insect pests, and synthetic biology.
- ***Ecosystem Processes*** includes opportunities related to soil carbon capture and sequestration, greenhouse gas mitigation, albedo change, hydrology, water quality, and biodiversity services.
- ***Multiscale Modeling*** connects and integrates these opportunities across multiple spatial and temporal scales to form a predictive understanding of sustainability outcomes over a range of future climate and management scenarios.

Fully addressing these research opportunities will require new approaches, tools, and a sustained commitment for supporting the long-term experimentation necessary for successful outcomes. Among these needs are replicated field sites; advanced instrumentation including sensors, *in situ* imaging, and coordinated phenotyping platforms; and a cyberinfrastructure that enables organization of data from multiple sources and provides new and more user friendly software tools, along with adequate computational capacity. Finally, full implementation of these approaches will require workforce training to provide the next generation of biofuel system scientists with the foundational and interdisciplinary knowledge needed to address complex systems-level challenges.

Appendix A: Agenda

Research for Sustainable Bioenergy Workshop

October 2–4, 2013

Day 1: Wednesday, Oct. 2, 2013

7:30 a.m.–8:30 a.m.	Arrival at Germantown campus and badging
8:30 a.m.–8:45 a.m.	Welcome, introduction, and overview by DOE BER program representatives and co-chairs
8:45 a.m.–9:00 a.m.	Participant introductions
Plenary Session:	Three brief introductory talks focusing on plant, microbial, and ecosystem aspects
9:00 a.m.–9:30 a.m.	**Plenary I:** Jonathan Lynch, Pennsylvania State University *"Resource Use Efficiency in Plants: Challenges and Opportunities"*
9:30 a.m.–10:00 a.m.	**Plenary II:** Mary Firestone, University of California, Berkeley *"Sustainability is in the Soil"*
10:00 a.m.–10:15 a.m.	Break
10:15 a.m.–10:45 a.m.	**Plenary III:** Evan DeLucia, University of Illinois at Urbana-Champaign *"Biogeochemistry of Bioenergy Driven Land Use Change: Promise and Challenge"*
10:45 a.m.–11:30 a.m.	**Breakout I: Brainstorming**
11:30 a.m.–12:15 p.m.	**General discussion** — Are we asking the right questions?
12:15 p.m.–1:00 p.m.	Lunch
1:00 p.m.–5:10 p.m.	**Breakout II: Identify key knowledge gaps and opportunities**
1:00 p.m.–2:15 p.m.	**Breakout II-A: Sustainable biofuel crop improvement and environmental impacts**
2:15 p.m.–2:25 p.m.	Break
2:25 p.m.–3:40 p.m.	**Breakout II-B: Below- and aboveground processes**
3:40 p.m.–3:55 p.m.	Coffee (in breakout rooms)
3:55 p.m.–5:10 p.m.	**Breakout II-C: Environmental effects/factors**
5:10 p.m.–6:00 p.m.	**Reports from breakout groups:** 10-minute summary per group, no slides

Day 2: Thursday, Oct. 3, 2013

7:30 a.m.–8:00 a.m.	Arrival at Germantown campus
8:00 a.m.–9:30 a.m.	**Breakout III: Summary — Bridging molecular and ecosystem studies**
9:30 a.m.–10:00 a.m.	Break
10:00 a.m.–11:30 a.m.	**Presentations from the breakout groups** (30 minutes each)
11:30 a.m.–12:30 p.m.	**Discussion and wrap-up**
12:30 p.m.	Participants adjourn
12:30 p.m.	Co-chairs, breakout leads, and DOE BER staff: lunch/discussion, writing session

Day 3: Friday, Oct. 4, 2013

Writing session:	Co-chairs, breakout leads

Appendix B: Workshop Participants

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*Unable to attend workshop but contributed to report.

Michael Casler, U.S. Department of Agriculture, also contributed to this report.

Report preparation: Biological and Environmental Research Information System group at Oak Ridge National Laboratory (Kris Christen, Holly Haun, Brett Hopwood, Betty Mansfield, Sheryl Martin, Marissa Mills, and Judy Wyrick)

Appendix C: References

Abril, A. B., P. A. Torres, and E. H. Bucher. 2005. "The Importance of Phyllosphere Microbial Populations in Nitrogen Cycling in the Chaco Semi-Arid Woodland," *Journal of Tropical Ecology* **21**(1), 103–107. DOI:10.1017/S0266467404001981.

Ashley, M. V. 2010. "Plant Parentage, Pollination, and Dispersal: How DNA Microsatellites Have Altered the Landscape," *Critical Reviews in Plant Sciences* **29**, 148–61. DOI:10.1080/07352689.2010.481167.

Bailey, V. L., et al. 2012. "Measurements of Microbial Community Activities in Individual Soil Macroaggregates," *Soil Biology and Biochemistry* **48**, 192–95. DOI:10.1016/j.soilbio.2012.01.004.

BERAC. 2013. *BER Virtual Laboratory: Innovative Framework for Biological and Environmental Grand Challenges; A Report from the Biological and Environmental Research Advisory Committee*, DOE/SC-0156. science.energy.gov/ber/berac/reports/.

Blagodatskaya, E., and Y. Kuzyakov. 2008. "Mechanisms of Real and Apparent Priming Effects and Their Dependence on Soil Microbial Biomass and Community Structure: Critical Review," *Biological and Fertility of Soils* **45**(2), 115–31. DOI:10.1007/s00374-008-0334-y.

Blum, A. 2005. "Drought Resistance, Water-Use Efficiency, and Yield Potential — Are They Compatible, Dissonant, or Mutually Exclusive?" *Australian Journal of Agricultural Research* **56**(11), 1159–68. DOI:10.1071/AR05069.

Blum, A. 2009. "Effective Use of Water (EUW) and Not Water-Use Efficiency (WUE) is the Target of Crop Yield Improvement Under Drought Stress," *Field Crops Research* **112**(s 2–3), 119–23. DOI:10.1016/j.fcr.2009.03.009.

Boddey, R. M., et al. 2003. "Endophytic Nitrogen Fixation in Sugarcane: Present Knowledge and Future Applications," *Plant and Soil* **252**(1), 139–49. DOI:10.1023/A:1024152126541.

Cameron, D. D., et al. 2013. "Mycorrhiza-Induced Resistance: More than the Sum of Its Parts?" *Trends in Plant Science* **18**(10), 539–45. DOI:10.1016/j.tplants.2013.06.004.

Chaparro, J. M., D. V. Badri, and J. M. Vivanco. 2014. "Rhizosphere Microbiome Assemblage is Affected by Plant Development," *The ISME Journal* **8**(4), 790–803. DOI:10.1038/ismej.2013.196.

Chapman, M. A., and J. M. Burke. 2006. "Letting the Gene Out of the Bottle: The Population Genetics of Genetically Modified Crops," *New Phytologist* **170**(3), 429–43. DOI:10.1111/j.1469-8137.2006.01710.x.

Clark, R. B., S. K. Zeto, and R. W. Zobel. 1999. "Arbuscular Mycorrhizal Fungi Isolate Effectiveness on Growth and Root Colonization of *Panicum Virgatum* in Acidic Soils," *Soil Biology and Biochemistry* **31**(13), 1757–63. DOI:10.1016/S0038-0717(99)00084-X.

Clifton-Brown, J. C., and I. Lewandowski. 2000. "Water Use Efficiency and Biomass Partitioning of Three Different *Miscanthus* Genotypes with Limited and Unlimited Water Supply," *Annals of Botany* **86**(1), 191–200. DOI:10.1006/anbo.2000.1183.

Condon, A., et al. 2004. "Breeding for High Water-Use Efficiency," *Journal of Experimental Botany* **55**(407), 2447–60. DOI:10.1093/jxb/erh277.

Davinic, M., et al. 2012. "Pyrosequencing and Mid-Infrared Spectroscopy Reveal Distinct Aggregate Stratification of Soil Bacterial Communities and Organic Matter Composition," *Soil Biology and Biochemistry* **46**, 63-72.

Davis, S. C., et al. 2010. "Comparative Biogeochemical Cycles of Bioenergy Crops Reveal Nitrogen-Fixation and Low Greenhouse Gas Emissions in a *Miscanthus* × *giganteus* Agro-Ecosystem," *Ecosystems* **13**, 144–56. DOI:10.1007/s10021-009-9306-9.

DeAngelis, K. M., et al. 2009. "Selective, Progressive Response of Soil Microbial Community to Wild Oat Roots," *The ISME Journal* **3**(2), 168–78. DOI:10.1038/ismej.2008.103.

Del Grosso, S. J., et al. 2000. "General CH_4 Oxidation Model and Comparisons of CH_4 Oxidation in Natural and Managed Systems," *Global Biogeochemical Cycles* **14**(4), 999–1019. DOI:10.1029/1999GB001226.

DiFazio, S. P., et al. 2012. "Gene Flow and Simulation of Transgene Dispersal from Hybrid Poplar Plantations," *New Phytologist* **193**(4), 903–15. DOI:10.1111/j.1469-8137.2011.04012.x.

Ellstrand, N. C., and K. A. Schierenbeck. 2000. "Hybridization as a Stimulus for the Evolution of Invasiveness in Plants?" *Proceedings of the National Academy of Sciences USA* **97**(13), 7043–50. DOI:10.1073/pnas.97.13.7043.

Erickson, J. E., et al. 2012. "Water Use and Water-Use Efficiency of Three Perennial Bioenergy Grass Crops in Florida," *Agriculture* **2**(4), 325–38. DOI:10.3390/agriculture2040325.

Flexas, J., et al. 2006. "Keeping a Positive Carbon Balance Under Adverse Conditions: Responses of Photosynthesis and Respiration to Water Stress," *Physiologia Plantarum* **127**(3), 343–52. DOI:10.1111/pp1.2006.127.issue-3/issuetoc.

Gelfand, I., et al. 2013. "Sustainable Bioenergy Production from Marginal Lands in the US Midwest," *Nature* **493**(7433), 514–17. DOI:10.1038/nature11811.

Gelfand, I., et al. 2011. "Carbon Debt of Conservation Reserve Program (CRP) Grasslands Converted to Bioenergy Production," *Proceedings of the National Academy of Sciences USA* **108**(33), 13864–69. DOI:10.1073/pnas.1017277108.

Ghimire, S. R., N. D. Charlton, and K. D. Craven. 2009. "The Mycorrhizal Fungus, *Sebacina vermifera*, Enhances Seed Germination and Biomass Production in Switchgrass (*Panicum virgatum* L)," *Bioenergy Research* **2**(1–2), 51–58. DOI:10.1007/s12155-009-9033-2.

Grattapaglia, D., et al. 2009. "Genomics of Growth Traits in Forest Trees," *Current Opinion in Plant Biology* **12**(2), 148–56. DOI:10.1016/j.pbi.2008.12.008.

Gyaneshwar, P., et al. 2011. "Legume-Nodulating Betaproteobacteria: Diversity, Host Range, and Future Prospects," *Molecular Plant-Microbe Interactions* **24**(11), 1276–88. DOI:10.1094/MPMI-06-11-0172.

Harrison, M. J. 2005. "Signaling in the Arbuscular Mycorrhizal Symbiosis," *Annual Reviews of Microbiology* **59**, 19–42. DOI:10.1146/annurev.micro.58.030603.123749.

Hodge, A., and A. H. Fitter. 2010. "Substantial Nitrogen Acquisition by Arbuscular Mycorrhizal Fungi from Organic Material has Implications for N Cycling," *Proceedings of the National Academy of Sciences USA* **107**(31), 13754–59. DOI:10.1073/pnas.1005874107.

Inselsbacher, E., et al. 2011. "The Potential of Microdialysis to Monitor Organic and Inorganic Nitrogen Compounds in Soil," *Soil Biology and Biochemistry* **43**(6), 1321–32. DOI:10.1016/j.silbio.2011.03.003.

Jansa, J., P. Bukovska, and M. Gryndler. 2013. "Mycorrhizal Hyphae as Ecological Niche for Highly Specialized Hypersymbionts—or Just Soil Free-Riders?" *Frontiers in Plant Science* **4**(Article 134), 1–8. DOI:10.3389/fpls.2013.00134.

Jastrow, J. D., J. E. Amonette, and V. L. Bailey. 2007. "Mechanisms Controlling Soil Carbon Turnover and Their Potential Application for Enhancing Carbon Sequestration," *Climatic Change* **80**, 5–23. DOI:10.1007/s10584-006-9178-3.

Jastrow, J. D., and R. M. Miller. 1998. "Soil Aggregate Stabilization and Carbon Sequestration: Feedbacks Through Organomineral Associations." In *Soil Processes and the Carbon Cycle*. 207–23. Eds. R. Lal, R. F. Follett, and B. A. Stewart. CRC Press LLC, Boca Raton, Florida.

Javot, H., et al. 2007. "A *Medicago truncatula* Phosphate Transporter Indispensable for the Arbuscular Mycorrhizal Symbiosis," *Proceedings of the National Academy of Sciences USA* **104**(5), 1720–25. DOI:10.1073/pnas.0608136104.

Kakani, V. G., et al. 2011. "Leaf Photosynthesis and Carbohydrates of CO_2-Enriched Maize and Grain Sorghum Exposed to a Short Period of Soil Water Deficit During Vegetative Development," *Journal of Plant Physiology* **168**(18), 2169–76. DOI:10.1016/j.jplph.2011.07.003.

Kaplan, D., et al. 2013. "A Survey of the Microbial Community in the Rhizosphere of Two Dominant Shrubs of the Negev Desert Highlands, *Zygophyllum dumosum* (Zygophyllaceae) and *Atriplex halimus* (Amaranthaceae), Using Cultivation-Dependent and Cultivation-Independent Methods," *American Journal of Botany* **100**(9), 1713–25. DOI:10.3732/ajb.1200615.

King, G. M. 2011. "Enhancing Soil Carbon Storage for Carbon Remediation: Potential Contributions and Constraints by Microbes," *Trends in Microbiology* **19**(2), 75–84. DOI:10.1016/j.tim.2010.11.006.

Knoth, J. L., et al. 2014. "Biological Nitrogen Fixation and Biomass Accumulation Within Poplar Clones as a Result of Inoculations with Diazotrophic Endophyte Consortia," *New Phytologist* **201**(2), 599–609. DOI:10.1111/nph.12536.

Kravchenko, A., et al. 2013. "Relationships Between Intra-Aggregate Pore Structures and Distributions of *Escherichia coli* Within Soil Macro-Aggregates," *Applied Soil Ecology* **63**, 134–42. DOI:10.1016/j.apsoil.2012.10.001.

Kuparinen, A., and F. M. Schurr. 2007. "Assessing the Risk of Gene Flow from Genetically Modified Trees Carrying Mitigation Transgenes," *Biological Invasions* **10**(3), 281–90. DOI:10.1007/s10530-007-9129-6.

Kwit, C., and C. N. Stewart. 2012. "Gene Flow Matters in Switchgrass (*Panicum virgatum* L.), a Potential Widespread Biofuel Feedstock," *Ecological Applications* **22**(1), 3–7. DOI:10.1890/11-1516.1.

Leakey, A., D. Ort, and S. Long. 2006. "Photosynthesis, Productivity, and Yield of Maize are not Affected by Open-Air Elevation of CO_2 Concentration in the Absence of Drought," *Plant Physiology* **140**(2), 779–90. DOI:10.1104/pp.105.073957.

Lynch, J. P. 2011. "Root Phenes for Enhanced Soil Exploration and Phosphorus Acquisition: Tools for Future Crops," *Plant Physiology* **156**(3), 1041–49. DOI:10.1104/pp.111.175414.

Lynch, J. P. 2013. "Steep, Cheap and Deep: An Ideotype to Optimize Water and N Acquisition by Maize Root Systems," *Annals of Botany* **112**(2), 347–57. DOI:10.1093/aob/mcs293.

Lynch, J. P., and K. M. Brown. 2012. "New Roots for Agriculture: Exploiting the Root Phenome," *Philosophical Transactions of the Royal Society of London, Biological Sciences* **367**(1595), 1598–1604. DOI:10.1098/rstb.2011.0243.

Mandyam, K., and A. Jumpponen. 2005. "Seeking the Elusive Function of the Root-Colonising Dark Septate Endophytic Fungi," *Studies in Mycology* **53**(1), 173–89. DOI:10.3114/sim.53.1.173.

Manschadi, A. M., et al. 2010. "Experimental and Modelling Studies of Drought-Adaptive Root Architectural Traits in Wheat (*Triticum aestivum* L.)," *Plant Biosystems* **144**(2), 458–62. DOI:10.1080/11263501003731805.

McCarthy, M. I., et al. 2008. "Genome-Wide Association Studies for Complex Traits: Consensus, Uncertainty and Challenges," *Nature Reviews Genetics* **9**(5), 356–69. DOI:10.1038/nrg2344.

Meirmans, P. G., J. Bousquet, and N. Isabel. 2009. "A Metapopulation Model for the Introgression from Genetically Modified Plants into Their Wild Relatives," *Evolutionary Applications* **2**(2), 160–71. DOI:10.1111/j.1752-4571.2008.00050.x.

Mendes R., P. Garbeva, and J. M. Raaijmakers. 2013. "The Rhizosphere Microbiome: Significance of Plant Beneficial, Plant Pathogenic, and Human Pathogenic Microorganisms," *Federation of European Microbiological Studies (FEMS) Microbiology Reviews* **37**(5), 634–63. DOI:10.1111/1574-6976.12028.

Meuwissen, T. H. E., B. J. Hayes, and M. E. Goddard. 2001. "Prediction of Total Genetic Value Using Genome-Wide Dense Marker Maps," *Genetics* **157**(4), 1819–29.

Mutava, R. N., et al. 2011. "Characterization of Sorghum Genotypes for Traits Related to Drought Tolerance," *Field Crops Research* **123**(1), 10–18. DOI:10.1016/j.fcr.2011.04.006.

Nagler, P. L., et al. 2011. "Distribution and Abundance of Saltcedar and Russian Olive in the Western United States," *Critical Reviews in Plant Sciences* **30**(6), 508–23. DOI:10.1080/07 5352689.2011.615689.

Nair, S. S., et al. 2012. "Bioenergy Crop Models: Descriptions, Data Requirements and Future Challenges," *Global Change Biology: Bioenergy* **4**(6), 620–33. DOI:10.1111/j.1757-1707.2012.01166.x.

Newsham, K. K., A. H. Fitter, and A. R Watkinson. 1995. "Arbuscular Mycorrhiza Protect an Annual Grass from Root Pathogenic Fungi in the Field," *Journal of Ecology* **83**(6), 991–1000. DOI:10.2307/2261180.

Nuccio, E. E., et al. 2013. "An Arbuscular Mycorrhizal Fungus Significantly Modifies the Soil Bacterial Community and Nitrogen Cycling During Litter Decomposition," *Environmental Microbiology* **15**(6), 1870–81. DOI:10.1111/1462-2920.12081.

Oldroyd, G. E. D., and R. Dixon. 2014. "Biotechnological Solutions to the Nitrogen Problem," *Current Opinion in Biotechnology* **26**, 19–24. DOI:10.1016/j.copbio.2013.08.006.

Philippot, L., et al. 2013. "Going Back to the Roots: the Microbial Ecology of the Rhizosphere," *Nature Reviews Microbiology* **11**(11), 789–99. DOI:10.1038/nrmicro3109.

Pieruschka, R., and H. Poorter. 2012. "Phenotyping Plants: Genes, Phenes and Machines," *Functional Plant Biology* **39**(11), 813–20. DOI:10.1071/FPv39n11_IN.

Postma, J. A., and J. P. Lynch. 2012. "Complementarity in Root Architecture for Nutrient Uptake in Ancient Maize/Bean and Maize/Bean/Squash Polycultures," *Annals of Botany* **110**, 521–34. DOI:10.1093/aob/mcs082.

Power, A. G. 2010. "Ecosystem Services and Agriculture: Tradeoffs and Synergies," *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **365**(1554), 2959–71. DOI:10.1098/rstb.2010.0143.

Raghu, S., et al. 2006. "Adding Biofuels to the Invasive Species Fire?" *Science* **313**(5794), 1742. DOI:10.1126/science.1129313.

Ravindranath, N. H., et al. 2009. "Greenhouse Gas Implications of Land Use and Land Conversion to Biofuel Crops." In *Biofuels: Environmental Consequences and Interactions with Changing Land Use*. 111–25. Eds. R. W. Howarth and S. Bringezu. Scientific Committee on Problems of the Environment (SCOPE), Island Press, New York. cip.cornell.edu/scope/1245782006.

Robertson, G. P., et al. 2008. "Sustainable Biofuels Redux," *Science* **322**(5898), 49–50. DOI:10.1126/science.1161525.

Robertson, G. P., et al. 2011. "The Biogeochemistry of Bioenergy Landscapes: Carbon, Nitrogen, and Water Considerations," *Ecological Applications* **21**(4), 1055–67.

Ruiz-Lozano, J. M., R. Azcon, and M. Gomez. 1995. "Effects of Arbuscular-Mycorrhizal *Glomus* Species on Drought Tolerance: Physiological and Nutritional Plant Responses," *Applied and Environmental Microbiology* **61**(2), 456–60.

Ryan, R. P., et al. 2008. "Bacterial Endophytes: Recent Developments and Applications," *Federation of European Microbiological Societies: Microbiology Letters* **278**(1), 1–9. DOI:10.1111/j.1574-6968.2007.00918.x.

Sakai, A. K., et al. 2001. "The Population Biology of Invasive Species," *Annual Review of Ecology, Evolution, and Systematics* **32,** 305–32. DOI:1146/annurev.ecolsys.32.081501.114037.

Sanders, F. E., and P. B. Tinker. 1971. "Mechanism of Absorption of Phosphate from Soil by *Endogone* Mycorrhizas," *Nature* **233**, 278–79. DOI:10.1038/233278c0.

Schachtman, D. P., and J. Q. D. Goodger. 2008. "Chemical Root to Shoot Signaling Under Drought," *Trends in Plant Science* **13**(6), 281–87. DOI:10.1016/j.tplants.2008.04.003.

Scheublin, T. R., et al. 2010. "Characterisation of Microbial Communities Colonising the Hyphal Surfaces of Arbuscular Mycorrhizal Fungi," *The ISME Journal* **4**(6), 752–63. DOI:10.1038/ismej.2010.5.

Schlaeppi, K., et al. 2014. "Quantitative Divergence of the Bacterial Root Microbiota in *Arabidopsis thaliana* Relatives," *Proceedings of the National Academy of Sciences USA* **111**(2), 585–92. DOI:10.1073/pnas.1321597111.

Schoppach, R., and W. Sadok. 2012. "Differential Sensitivities of Transpiration to Evaporative Demand and Soil Water Deficit Among Wheat Elite Cultivars Indicate Different Strategies for Drought Tolerance," *Environmental Experimental Botany* **84**, 1–10. DOI:10.1016/j.envexpbot.2012.04.016.

Serebrovsky, A. S. 1925. " 'Somatic Segregation' in Domestic Fowl," *Journal of Genetics* **16**(1), 33–42. DOI:10.1007/BF02983986.

Sinclair, T. R. 2000. "Model Analysis of Plant Traits Leading to Prolonged Crop Survival During Severe Drought," *Field Crops Research* **68**(3), 211–17. DOI:10.1016/S0378-4290(00)00125-8.

Sinclair, T. R. 2012. "Is Transpiration Efficiency a Viable Plant Trait in Breeding for Crop Improvement?" *Functional Plant Biology* **39**(5), 359–65. DOI:10.1071/FP11198.

Singh, V., et al. 2012. "Genetic Control of Nodal Root Angle in Sorghum and Its Implications on Water Extraction," *European Journal of Agronomy* **42**, 3–10. DOI:10.1016/j.eja.2012.04.006.

Smith, S. E., and D. J. Read. 2008. *Mycorrhizal Symbiosis. Third Edition*. Academic Press, Elsevier, New York, NY.

Stockmann, U., et al. 2013. "The Knowns, Known Unknowns and Unknowns of Sequestration of Soil Organic Carbon," *Agriculture, Ecosystems and Environment* **164**, 80–99. DOI:10.1016/j.agee.2012.10.001.

Strauss, S. H. 2003. "Genomics, Genetic Engineering, and Domestication of Crops," *Policy Forum: Genetic Technologies. Science* **300**(5616), 61–62. DOI:10.1126/science.1079514.

Swinton, S. M., et al. 2007. "Ecosystem Services and Agriculture: Cultivating Agricultural Ecosystems for Diverse Benefits," *Ecological Economics* **64**(2), 245–52. DOI:10.1016/j.ecolecon.2007.09.020.

Tyler, H. L., and E. W. Triplett. 2008. "Plants as a Habitat for Beneficial and/or Human Pathogenic Bacteria," *Annual Review of Phytopathology* **46**, 53–73. DOI:10.1146/annurev.phyto.011708.103102.

Van der Heijden, M. G., R. D. Bardgett, and N. M. Van Straalen. 2008. "The Unseen Majority: Soil Microbes as Drivers of Plant Diversity and Productivity in Terrestrial Ecosystems," *Ecology Letters* **11**(3), 296–310. DOI:10.1111/j.1461-0248.2007.01139.x.

Van der Heijden, M. G., et al. 2006. "The Mycorrhizal Contribution to Plant Productivity, Plant Nutrition and Soil Structure in Experimental Grassland," *New Phytologist* **172**(4), 739–52. DOI:10.1111/j.1469-8137.2006.01862.x.

von Lützow, M., et al. 2006. "Stabilization of Organic Matter in Temperate Soils: Mechanisms and Their Relevance Under Different Soil Conditions—A Review," *European Journal of Soil Science* **57**(4), 426–45. DOI:10.1111/j.1365-2389.2006.00809.x.

Wang, Q., et al. 2013. "Ecological Patterns of *nifH* Genes in Four Terrestrial Climatic Zones Explored with Targeted Metagenomics Using Framebot, a New Informatics Tool," *mBio* **4**(5), E00592-13. DOI:10.1128/mBio.00592-13.

Warwick, S. I., H. J. Beckie, and L. M. Hall. 2009. "Gene Flow, Invasiveness, and Ecological Impact of Genetically Modified Crops," *Annals of the New York Academy of Sciences* **1168**, 72–99. DOI:10.1111/j.1749-6632.2009.04576.x.

Weill, M., et al. 2011. "Sebacinales Everywhere: Previously Overlooked Ubiquitous Fungal Endophytes," *PLoS ONE* **6**(2), e16793. DOI:10.1371/journal.pone.0016793.

Werling, B. P., et al. 2014. "Perennial Grasslands Enhance Biodiversity and Multiple Ecosystem Services in Bioenergy Landscapes," *Proceedings of the National Academy of Sciences USA* **111**, 1652–57. DOI:10.1073/pnas.1309492111.

West, T. O., and J. Six. 2007. "Considering the Influence of Sequestration Duration and Carbon Saturation on Estimates of Soil Carbon Capacity," *Climatic Change* **80**, 25–41. DOI:10.1007/s10584-006-9173-8.

Williams, C. G. 2010. "Long-Distance Pine Pollen Still Germinates After Meso-Scale Dispersal," *American Journal of Botany* **97**(5), 846–55. DOI:10.3732/ajb.0900255.

York, L. M., E. Nord, and J. Lynch. 2013. "Integration of Root Phenes for Soil Resource Acquisition," *Frontiers in Plant Science: Functional Plant Ecology* **4**(355). DOI:10.3389/fpls.2013.00355.

Zhang, X., et al. 2009. "Root Size, Distribution and Soil Water Depletion as Affected by Cultivars and Environmental Factors," *Field Crops Research* **114**(1), 75–83. DOI:10.1016/j.fcr.2009.07.006.

Zilber-Rosenberg, I., and E. Rosenberg. 2008. "Role of Microorganisms in the Evolution of Animals and Plants: The Hologenome Theory of Evolution," *Federation of European Microbiological Societies (FEMS) Microbiology Reviews* **32**(5), 723–35. DOI:10.1111/j.1574-6976.2008.00123.x.

Acronyms and Abbreviations

3D	three dimensional
AM	arbuscular mycorrhizal
BER	DOE Office of Biological and Environmental Research
BERAC	Biological and Environmental Research Advisory Committee
BNF	biological nitrogen fixation
C	carbon
CH_4	methane
CO_2	carbon dioxide
DOE	U.S. Department of Energy
EM	ectomycorrhizal
GE	genetically engineered
GFP	green fluorescent protein
GWAS	genome-wide association study
K	potassium
N	nitrogen
NEON	NSF National Ecological Observatory Network
NH_3	ammonia
N_2O	nitrous oxide
NSF	National Science Foundation
NUE	nitrogen use efficiency
O_2	oxygen
P	phosphorus
PET	positron emission tomography
SPECT	single photon emission computed tomography
TE	transpiration efficiency
USDA	U.S. Department of Agriculture
WUE	water use efficiency

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