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Resilient Crop Production for the Bioeconomy

Frontier Science for the Bioeconomy Workshop Series

U.S. DEPARTMENT of ENERGY | Office of Science
Biological and Environmental Research Program

Resilient Bioenergy Crop Production Workshop

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About BER

The Biological and Environmental Research (BER) program's mission is to support transformative science and scientific user facilities to achieve a predictive understanding of complex biological, Earth, and environmental systems. This research supports the U.S. Department of Energy's (DOE) vision to advance innovative solutions for U.S. energy expansion and national security challenges. BER's fundamental research, conducted at DOE national laboratories and other research institutions, plays a unique role in ensuring national leadership in biotechnology and the ability to understand and predict the interdependencies involving energy and the environment over a wide range of conditions.

Front cover image credit: Aerial roots of biomass sorghum, which can maintain high productivity under water limitation, are grown under experimental drought in a field at the University of Illinois Crop Science Research and Education Center in Savoy, Ill. [Courtesy William Eddy, University of Illinois Urbana-Champaign]

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Resilient Crop Production for the Bioeconomy

Workshop Report

Frontier Science for the Bioeconomy Workshop Series

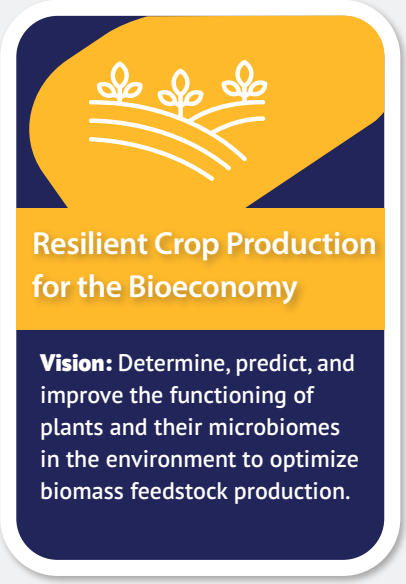
July 2026

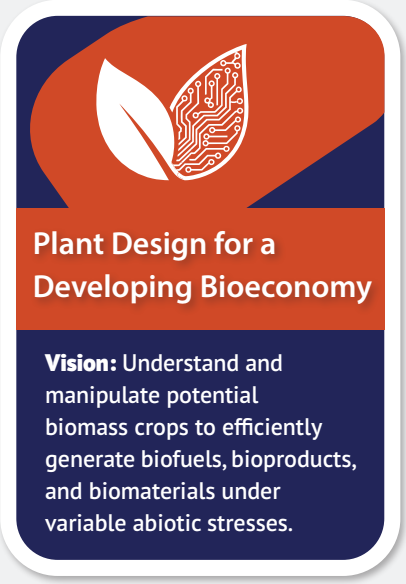
U.S. DEPARTMENT of ENERGY | Office of Science
Biological and Environmental Research Program

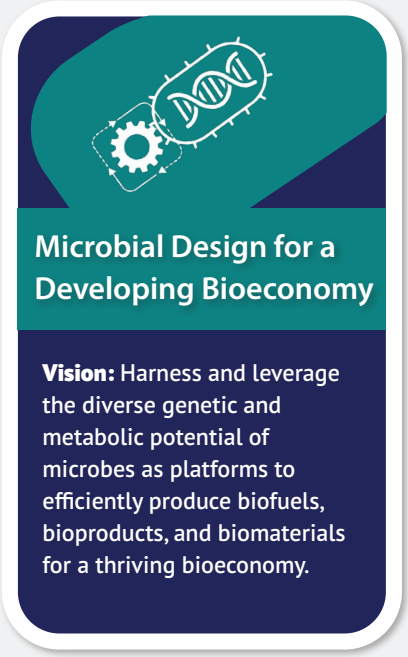
Frontier Science for the Bioeconomy

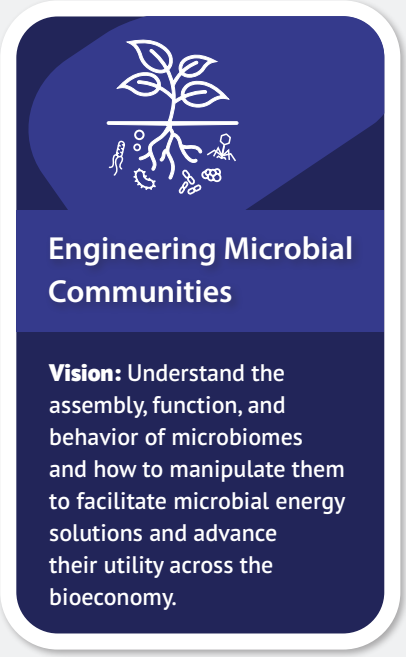
The *Resilient Crop Production for the Bioeconomy* workshop report is one of a four-part Frontier Science for the Bioeconomy series hosted by the DOE Biological and Environmental Research program. The series defines frontiers of plant science, agriculture, synthetic biology, biobased materials, and environmental microbiome science that will unlock the promise of an innovative, resilient U.S. bioeconomy.

The bioeconomy is economic activity driven by research and involving innovation in the life sciences, engineering, and biomanufacturing. It includes industries, products (e.g., chemicals, biomass and seed oil feedstocks, and bioproducts), services, and processes derived from plant and microbial resources.









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Executive Summary

A bioeconomy based on energy, chemicals, and bioproducts derived from nonfood crops promotes U.S. prosperity, energy independence, and national security. Crop productivity (i.e., yield) affects profitability for farmers, whereas biomass and seed oil quality affects profitability for biorefineries through the conversion efficiency of feedstock crops into energy, chemicals, and bioproducts. Scientific breakthroughs in plant genetics and genomics, ecological processes, bioprocessing, microbial engineering and design, and conversion technologies have improved the potential market viability of products derived from nonfood crops specifically cultivated as biomass and seed oil feedstocks.

Improving understanding of a plant's complex responses to withstand or recover from stress is crucial to maintaining feedstock yield and quality, particularly in challenging production zones. Stress may be related to biotic or abiotic (i.e., nonliving) environmental conditions that negatively affect plant growth, development, and productivity. Research and development to enhance the environmental resilience of feedstock crops will enable improved production by minimizing losses during stress and improving production on currently economically nonviable marginal lands.

About the Workshop

The U.S. Department of Energy (DOE) Biological and Environmental Research (BER) program seeks predictive understanding of complex biological and environmental systems. Within BER, the Biological Systems Science Division (BSSD)[1] aims to leverage a basic understanding of plants and their associated microbial communities to advance feedstock production for fuels, chemicals, and other bioproducts in support of a burgeoning bioeconomy. To explore research opportunities and strategies in this area, BER held a virtual Resilient Bioenergy Crop Production workshop from October 30 through November 1, 2024. The workshop convened 66 participants from academia, industry, and DOE national laboratories spanning expertise in plant physiology, plant genetics, genomics, computational biology, modeling, microbiology, ecology, and agronomy. Breakout topics were organized around current and emerging nonfood industrial crops (i.e., trees, perennial and annual grasses, and oilseed crops), growing regions across the United States, and abiotic stressors (i.e., nutrient limitation, drought, salinity, cold, and heat). Participants discussed environmental challenges, solutions, and key knowledge gaps in resilient crop production for a robust bioeconomy, as well as research opportunities to fill knowledge gaps and develop new solutions.

Resilient crops support a vibrant bioeconomy by providing consistent production across environmental conditions and expanded production opportunities across the United States.

The workshop was one of a four-part Frontier Science for the Bioeconomy series hosted by BSSD. The series defines frontiers of plant science, agricultural sustainability, synthetic biology, biobased materials, and environmental microbiome science that can unlock an innovative, resilient U.S. bioeconomy.

Pursuing Crop Resilience

Participants defined resilient crop production as the ability of a production system to provide consistent

[1] BSSD has now been reorganized as part of two new BER divisions.

yields and feedstock composition, as well as ecosystem processes that sustain long-term system productivity, despite abiotic and biotic stressors. This ability can be derived from plant traits, plant–microbe interactions, and cropping system design, and manifests as stressor avoidance, acclimation, and adaptation strategies.

Stress can differ in severity and duration—from low severity that reduces crop productivity to high severity that threatens crop survival and from short-term acute stress to long-term chronic stress. The concept of crop resilience encompasses responses not only to short-term perturbations but also chronic stress (e.g., found in nutrient-poor soils or water-limited growing regions), stressors that develop over time (e.g., progressive soil degradation during long-term cultivation), and loss of vigor (e.g., aging perennial crops).

Although outside the workshop scope, economic resilience was recognized as critically important in driving farmers' adoption of crops grown as biomass or bioenergy feedstocks. Typically, such crops are grown with low agricultural inputs and under long-term contracts with biomass purchasers, both of which can reduce financial risks for farmers overexposed to fluctuating market prices and input costs of traditional crops. Providing farmers with access to capital to cover the lag between planting and peak biomass yield is likely to encourage farmer adoption. Dedicated feedstock crops are currently excluded from public crop support programs and private agricultural lending products that could facilitate their incorporation into existing crop enterprises, particularly by reducing the burden of front-loaded establishment costs and crop-specific investments for perennials.

In locations where feedstock crops exhibit greater resilience against abiotic and biotic stressors than conventional crops, transitioning land to feedstock crop production can increase profitability and decrease financial risk for farmers. Several opportunities exist to expand resilient feedstock crop production without negatively impacting conventional crop production, including (1) integrating feedstock crops into conventional cropping systems, (2) extending crop production into new growing zones, (3) reclaiming less productive conventional cropland, and (4) improving crop resilience on marginal lands. Improving the resilience of feedstock cropping systems therefore not only benefits production on existing feedstock croplands but also supports rural development and bioeconomic growth by expanding opportunities for profitable feedstock crop production.

Key Knowledge Gaps and Research Opportunities

Abiotic stress response mechanisms in plants differ considerably by stressor type and crop species. Similarly, current understanding of mechanisms underlying beneficial plant–microbe interactions differs by stressor type and microbial functional group. This report identifies key knowledge gaps and research opportunities to broadly enable engineering of more resilient feedstock crops as well as beneficial plant–microbe interactions (see Fig. ES.1, p. ix). These gaps and opportunities are organized into four categories: (1) plants, including current and emerging feedstock crops; (2) plant microbiomes, including plant-recruited microbes and amended bioinoculants; (3) cropping systems, including spatially and temporally diverse systems; and (4) crosscutting resources, including shared resources and facilities, new instrumentation, modeling, and workforce development.

Plant Resilience to Abiotic Stress

Feedstock crop productivity and composition is strongly influenced by resource availability and the interplay of environmental factors across a crop's life cycle. Abiotic stressors, including water availability and temperature extremes, low soil fertility, and high salinity, are key factors constraining productivity and feedstock quality both individually and in combination. Selecting crop species or varieties adapted to local environmental conditions becomes more difficult as these conditions change, especially with multiple interacting stressors. Better understanding of fundamental stress response mechanisms can guide biodesign and development of genotypes with improved performance under field conditions. To facilitate scaling to real-world conditions, model and nonmodel feedstock crop species need

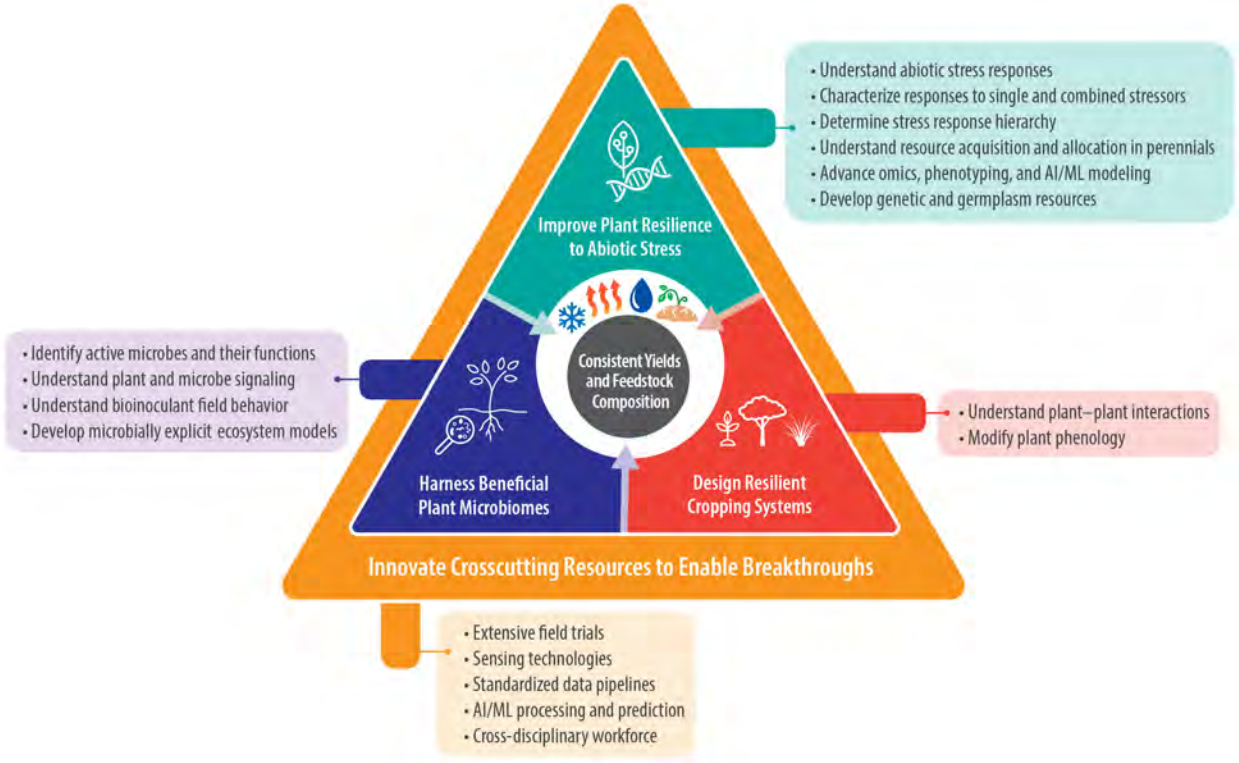

Fig. ES.1. Key Knowledge Gaps and Research Opportunities in Resilient Feedstock Crop Production. Priority research opportunities that will provide unprecedented opportunity for resilient cropping systems, featuring consistent yields and feedstock composition, include improving resilience traits in crop species, harnessing the beneficial plant microbiome, designing more resilient cropping systems, and developing crosscutting innovations in technologies and resources to enable these breakthroughs.

to be studied under realistic stress conditions in controlled environments, as well as across diverse field environments.

Priority research opportunities to address these plant knowledge gaps include:

- Improve understanding of genetic, molecular, and physiological processes governing plant responses to abiotic stress.
- Characterize plant responses to individual stressors and combinations of stressors across developmental stages and plant organizational scales.
- Determine the hierarchy of plant stress response processes and their interconnectedness across different levels of biological organization.
- Characterize physiological and molecular bases of seasonal and interannual growth, nutrient storage, and remobilization patterns of perennial species.
- Advance omics, phenotyping, and artificial intelligence and machine learning (AI/ML)–assisted modeling to better understand stress response mechanisms and guide feedstock crop improvement.
- Develop and preserve genetic and germplasm resources for breeding, genome editing, and mechanistic studies.

Beneficial Plant Microbiomes

Interactions between plants and the microbes living within, on, and around them can benefit plant

Advances in artificial intelligence applied to designing resilient feedstock crops and improved microbiomes will accelerate and enable biotechnology breakthroughs.

productivity and resilience. However, the ability to manipulate and leverage these interactions to increase crop resilience is limited by an insufficient mechanistic understanding of how microbes provide these benefits to plants and which microbes persist and actively provide beneficial functions under stress conditions. Bioinoculation of plants with beneficial individual microbes or consortia currently lacks proof of concept due to the uncharacterized mechanisms underlying beneficial microbial functions, difficulty getting bioinoculants to persist in the field, and concerns about containing bioinoculants released in the field.

Priority research opportunities to address these plant microbiome knowledge gaps include:

- In the context of the phytobiome, identify and characterize active microbes and their beneficial functions that are relevant to crop resilience under abiotic stress.
- Determine plant and microbial signaling mechanisms that govern microbiome selection and plant form and function by leveraging advances in omics technologies and AI/ML models.
- Determine mechanisms of bioinoculant function, persistence, and containment.
- Develop microbially explicit models that connect microbial processes to large-scale models.

Resilient Cropping Systems

In addition to cropping systems benefiting from improved resilience in individual cultivars or crop species, further gains can be achieved by introducing cultivar and species diversity over spatial or time scales. These strategies are generally faster to deploy than engineered crop improvements or microbiomes, creating nearer-term opportunities to enhance crop resilience at scale.

While diverse species plantings, polyclonal plantings, intercropping, and nurse plantings have benefited crop productivity and resilience in some cases, a lack of fundamental understanding about plant–plant interactions for feedstock crops has limited the ability to design spatially diverse cropping systems. Efforts to temporally diversify cropping systems, via double cropping or cover cropping, have been constrained by fitting the life cycle of productive intermediate crops into the periods between primary cash crops.

Priority research opportunities to address cropping system knowledge gaps include:

- Improve understanding of plant–plant interactions to enable design of cropping systems that use spatial diversity and temporal complementarity to confer resilience.
- Modify phenology of intermediate crops to optimize their harvest and subsequent planting of primary cash crops.

Crosscutting Resources To Enable Breakthroughs

Feedstock crop production results from a complex web of interactions among plants, microbes, soil processes, and environmental conditions that challenge efforts to predict how crop productivity and composition will respond to stressors. In addition, the timing of environmental stressors determines their impact severity on crop production. While investigations of simplified, controlled systems are needed to advance knowledge, field studies are imperative to test plant and microbial mechanisms under complex, real-world conditions. Developing new technologies and expanding shared resources and infrastructure are critical to accelerate progression of fundamental and translational research from molecular to field scales. Improved modeling capabilities, including AI/ML approaches and new algorithms, are needed to disentangle these complexities and accelerate development of resilient cropping systems. Robust datasets integrating

above- and belowground traits and processes of plants and microbes require innovations and deployment of advanced omics and sensing tools.

Crosscutting research needs to enable progress on plant and microbiome research opportunities include:

- Conduct long-term, temporally resolved, multi-location field trials spanning a broad range of environmental conditions to extend and validate controlled-environment research and to generalize knowledge across time and space.
- Develop high-throughput, complementary sensing modalities for plants and microbes that, together with comprehensive environmental monitoring, facilitate modeling and predictive plant breeding and engineering strategies.
- Create standardized data acquisition and processing pipelines to enable data synthesis across studies and data integration into current and emerging AI/ML applications.
- Innovate AI/ML approaches to leverage datasets generated by advanced omics technologies and high-throughput phenotyping platforms; reduce data burdens to untangle the effects of genetic, environmental, and management (G x E x M) interactions on plant, microbial, and soil processes; and create computationally efficient surrogates for large-scale models.
- Train a cross-disciplinary workforce for the bioeconomy, including providing training in applied AI/ML for use in feedstock crop research.

Recent advances in biotechnology, omics technologies, phenotyping sensors, isotope tracing, and AI/ML are already accelerating research progress in understanding how plants respond to abiotic stressors and interact with beneficial microbes. Further innovations in instrumentation (e.g., high-throughput sensing technologies) and computational tools (e.g., AI/ML-aided integration of plant and microbial data across spatial, temporal, and biological scales) will transform capabilities to identify key plant and microbial traits that can be predictably engineered or managed. The development of shared research infrastructure, including field sites spanning broad environmental gradients for testing G x E x M, will play a critical role in tailoring cultivars, microbiomes, and management practices for specific production environments across the United States. Investment in key research opportunities will provide unprecedented opportunity for resilient cropping systems to serve as reliable feedstock sources for a vibrant bioeconomy that safeguards national security and energy independence while promoting rural development and economic prosperity.

Chapter 1

Resilient Feedstock Crops for a Robust Bioeconomy

Decades of federal research investment in producing energy, chemicals, and bioproducts from nonfood crops have led to major scientific advances, forming the foundation for a growing bioeconomy that promotes U.S. economic prosperity, energy independence, and national security. Scientific breakthroughs in plant genetics and genomics, ecological processes, bioprocessing, and conversion technologies have improved the potential market viability of energy, chemicals, and bioproducts derived from cellulosic feedstocks. Research on dedicated feedstock crops that are productive on marginal lands has shown promise in expanding the bioeconomy and creating opportunities for rural communities. The economic stability required to de-risk movement toward a cellulosic feedstock–based bioeconomy relies on consistent crop production (i.e., yield) in the face of abiotic and biotic stressors.

To explore research opportunities and strategies in these areas, the U.S. Department of Energy (DOE) Biological and Environmental Research program convened a virtual Resilient Bioenergy Crop Production workshop from October 30 through November 1, 2024 (see Appendix A: Workshop Agenda, p. 67). The workshop included 66 invited participants with expertise in current and emerging dedicated feedstock crops (e.g., trees, perennial and annual grasses, and oilseed crops) representing multiple growing regions across the United States (see Appendix B: Workshop Participants, p. 70). The workshop consisted of plenary presentations and breakout sessions covering:

- Environmental challenges in resilient feedstock crop production.
- Solutions to environmental challenges in resilient feedstock crop production.
- Key knowledge gaps in resilient feedstock crop production for a sustainable bioeconomy.
- Research opportunities to fill key knowledge gaps and develop new solutions.

Workshop participants completed a pre-workshop survey to focus the breakout sessions on abiotic stressors they considered most important—drought, flood, nutrient limitation, salinity, heat, and cold. Biotic stress was not directly covered in this workshop. The pre-workshop survey also identified participants' primary and secondary areas of expertise (i.e., plants, microbiomes, and ecosystems) and specific interest in crop type, plant species, and growing region. These selections were used to assign participants to breakout groups to focus discussion within specific contexts and answer questions efficiently. Some participants answered different sets of questions based on their area of expertise (see Appendix C: Breakout Questions, p. 72). This report summarizes the main conclusions from group discussions and highlights critical research needs and opportunities for resilient feedstock crop production.

1.1 Defining Crop Resilience

Abiotic stressors are nonliving environmental conditions that can negatively impact plant growth, development, and productivity. Improved understanding of the complex plant responses required to withstand or recover from stress is crucial to maintaining feedstock

Resilient Feedstock Crop Production
The ability of a crop production system to provide consistent yields and feedstock composition, as well as ecosystem processes that sustain long-term system productivity, despite abiotic and biotic stressors.

yield, particularly in challenging production conditions. To this end, a consensus definition of resilient feedstock crop production set the context for workshop discussions: The ability of a feedstock crop production system to provide consistent yields and feedstock composition, as well as ecosystem processes that sustain long-term system productivity, despite abiotic and biotic stressors. This ability of crops can be derived from plant traits, plant–microbe interactions, and cropping system design, and manifests as stressor avoidance, acclimation, or adaptation strategies.

Resilience is related to the rate at which a system returns to baseline after perturbation and is one of many properties of cropping system stability. Other properties contributing to system stability include resistance—the ability to withstand changes caused by perturbations; tolerance—the ability to tolerate perturbations without shifting into an alternative stable state; and recovery—the ability to recover fully from a perturbation (Van Meerbeek et al. 2021).

In this report, resilience is shorthand, referring broadly to these various potential attributes of a resilient crop production system. Stress can differ in severity and duration, from short-term acute stress to long-term chronic stress (Lichtenhaler 1996). Here, the concept of system stability encompasses responses not only to short-term perturbations but also to chronic stress (e.g., found in nutrient-poor soils or water-limited growing regions), stressors that develop over time (e.g., progressive soil degradation during long-term cultivation), and loss of vigor (e.g., aging perennial crops). Severe perturbations that test the limits of stress tolerance, threatening not just crop growth but crop survival, are likely to occur infrequently. Therefore, resilience against such perturbations or abiotic stressors, while important, may be considered lower priority than resilience against less severe perturbations.

Although outside the workshop scope, economic resilience is paramount to driving farmers' adoption of dedicated feedstock crops. Nonfood feedstock crops typically are grown with low agricultural inputs and under long-term contracts with biomass purchasers, both of which can reduce financial risks for farmers overexposed to fluctuating market prices and input costs of traditional crops (Vos et al. 2025). Providing farmers with access to capital that bridges the lag between planting and peak biomass yield is likely to encourage farmer adoption. Dedicated feedstock crops are currently excluded from public crop support programs and private agricultural lending products that could facilitate their incorporation into existing crop enterprises, particularly by reducing the burden of front-loaded establishment costs and crop-specific investments for perennials (Fewell et al. 2016; Khanna et al. 2017; Burli et al. 2021). Furthermore, cultivating feedstock crops that protect and restore soil health can buffer against catastrophic yield and financial losses well into the future (Kane et al. 2021).

1.2 Environmental Challenges in Feedstock Crop Production

Feedstock production is the foundation of the bioeconomy, constraining the value, volume, quality, consistency, and sustainability of energy, chemicals, and bioproducts generated by downstream processes (Somerville et al. 2010). Environmental factors fundamentally constrain crop productivity and quality (Lobell et al. 2009), with potentially far-reaching consequences for economic growth, domestic supply chains, biosecurity, and U.S. competitiveness internationally. Therefore, high-priority research topics include understanding and improving feedstock crop resilience to environmental challenges.

Environmental factors that limit crop production, such as water, nutrients, temperature, and salt, vary in time and space (Churkina and Running 1998). At the global scale, drought stress—when water supply

fails to meet crop demand—limits crop production more than any other factor (Boyer 1982). Inadequate water supply can result from low total rainfall or high rainfall periodicity. High crop water demand can result from low humidity or high temperatures. Conversely, intense rainfall and poor drainage can cause flooding, which also drives significant crop losses or failure (Hu et al. 2020). Native soil nutrient supply—especially of nitrogen, phosphorus, and potassium—is frequently inadequate to maximize crop growth, so fertilizer applications have become ubiquitous in industrial agriculture (Mueller et al. 2012). High or low temperature extremes impair biological processes and can limit crop productivity by inducing stress during the growing season or shortening it (Schlenker and Roberts 2009; Xia et al. 2015). Saltwater intrusion and groundwater overuse can impair crop function by increasing soil salinity (Barlow and Reichard 2010).

Research and development to improve the environmental resilience of feedstock crops will enable improved production by minimizing losses during stress and improving production in currently economically nonviable marginal lands. In tandem with efforts to address specific environmental challenges, farmers critically need adapted plant species, genotypes, and cropping systems that can maintain productivity in the face of locally prevalent challenges. Several important aspects of environmental challenges to feedstock crop production include:

- **The timing of environmental stressors determines their impact severity on feedstock crop production.** Fluctuating temperatures affect both crop productivity and feedstock quality depending on the timing of cold snaps, heat waves, or drought relative to plant developmental stage. Frost events that occur after perennial crops break dormancy can reduce yields by delaying regrowth and can even cause mortality and stand losses (Sage et al. 2015). Perennial crops can exhibit long-term yield reductions, especially if a perturbation occurs during its establishment year (Clifton-Brown and Lewandowski 2000). High winds tend to cause lodging issues late in the growing season when plants (e.g., biomass sorghum) have grown tall enough to be susceptible.
- **Selecting feedstock crop species or varieties adapted to local environmental conditions becomes increasingly difficult as conditions evolve, especially in cases of multiple interacting stressors.** A common plant strategy for mitigating heat stress, for example, is to increase transpiration. However, heat waves combined with reduced rainfall can negate this strategy as plants close their stomata to minimize transpiration under water-limited conditions (Sinha et al. 2025). In the U.S. Southeast, saltwater intrusion can accompany flooding such that plants normally resilient to flooding may not survive the newly saline soil conditions. In addition, legacy effects of past disturbances, such as saltwater intrusion and soil erosion, can influence the long-term productivity of the land. Poor soil health can exacerbate the impacts of meteorological stressors on crop production on marginal lands.
- **Environmental changes can induce biotic stressors that impact feedstock crops.** Sudden increases in moisture can lead to pest and pathogen outbreaks, such as the spread of rust, smut, and anthracnose in switchgrass (Gravert et al. 2007; Layton and Bergstrom 2011; Uppalapati et al. 2013; Hoffman et al. 2016; Samson et al. 2016; Tian and Smith 2021; Bowen et al. 2022). Increasing temperature can alter pest life cycles or invite novel pathogen intrusions. For example, new diseases such as false smut are emerging in the U.S. Southeast (Willis et al. 2021). Although biotic stressors were not discussed during the workshop, the interaction between abiotic and biotic stressors cannot be overlooked.
- **Altered growing season length creates numerous challenges for feedstock crop production.** Growing season length determines planting and harvest dates of primary cash crops and can provide an opportunity to grow annual feedstock crops during the primary season. However, changes in season length in turn affect the planting and harvest timing of intermediate crops. A warm spring, for example, would lead to early cash crop planting. A mild winter that fails to freeze soils can delay the winter harvest, potentially impacting

both feedstock quality and regrowth, as well as perennial crop productivity in the next growing season. The photoperiod- and temperature-sensing interactions that occur in perennials control their release from dormancy during winter–spring transitions and impact flowering time, thereby affecting plant growth, fitness, and yields (Song et al. 2013; Singh et al. 2016). Warm winters can also affect vernalization at low latitudes, resulting in altered flowering timing. In addition, warm winters may impact soil nutrient cycling, necessitating changes in fertility management.

1.3 Expansion Opportunities in Feedstock Crop Production

Strategically integrating dedicated biomass and seed oil feedstock crops into arable agricultural systems is critical to protecting and enhancing U.S. energy, food, and manufacturing security. The 2023 Billion-Ton Report found the United States can harvest sufficient biomass to meet 15% of future energy needs without negatively affecting food production (U.S. DOE 2024a).

In most scenarios examined, herbaceous biomass crops provide at least 50% of all agricultural biomass, with agricultural residues providing the other 50%. In the mature-market medium scenario, in which market pull and supply push are both moderate, this equates to 345 million dry tons of biomass—99.6% of which is provided by key crops discussed in the workshop and includes, in part, switchgrass (67%), miscanthus (32%), and sorghum (0.1%).

Meeting this biomass goal will require dedicating a total of 76 million acres of cropland—equivalent to about 10% of existing agricultural land—to feedstock crops producing over 4.5 tons of biomass per acre. This percentage corresponds to the amount of underperforming cropland that, if converted to perennials, would result in 70 to 90% reductions in cropland soil erosion, trace gas emissions, and nutrient losses (Schulte et al. 2017) while increasing farm profits (Brandes et al. 2018).

Integrating dedicated feedstock crops into current cropping systems bolsters national security by developing energy, chemicals, and bioproducts manufacturing industries and enhances the natural resource base on which food security depends. In locations where feedstock crops exhibit greater resilience against abiotic and biotic stressors than conventional crops, transitioning the land to feedstock production can increase profitability and decrease financial risk for farmers. Several opportunities exist to expand resilient feedstock crop production without displacing conventional crop production (see Fig. 1.1, p. 5).

Integrating Feedstock Crops into Conventional Cropland

Opportunities exist for nonfood feedstock crops to complement, rather than compete with, conventional crops. Feedstock crops can be introduced into crop rotations to improve soil health, thereby increasing long-term cropping system resilience. For example, shorter-season feedstock crops, such as oilseed crops, could complement soybean or maize cycles and provide additional income to farmers (Basnet and Ellison 2024). Feedstock crops could also be integrated into multifunctional landscapes, such as buffer zones for flood control, erosion prevention, saltwater intrusion mitigation, and nutrient capture (Wang et al. 2020; Cooney et al. 2022; Schulte et al. 2022). Integrating perennial feedstock crops into unprofitable sections of conventional annual row-crop fields can both improve nutrient retention in the field and increase farm profitability (Brandes et al. 2017).

Expanding Production into New Growing Zones

Rising winter temperatures may extend the growing zone northward for some crops and may enable production of intermediate crops at lower latitudes during winter months. For example, yield gains have been achieved in several feedstock grasses by moving southern-adapted genotypes northward. In this case, the gains primarily result from increased growing season length due to photoperiod-driven delays in flowering transitions in the southern material (Casler 2012; Tilhou and Casler 2022). Typically, southern material is poorly suited to northern winters and can exhibit poor overwintering survival and low freezing tolerance

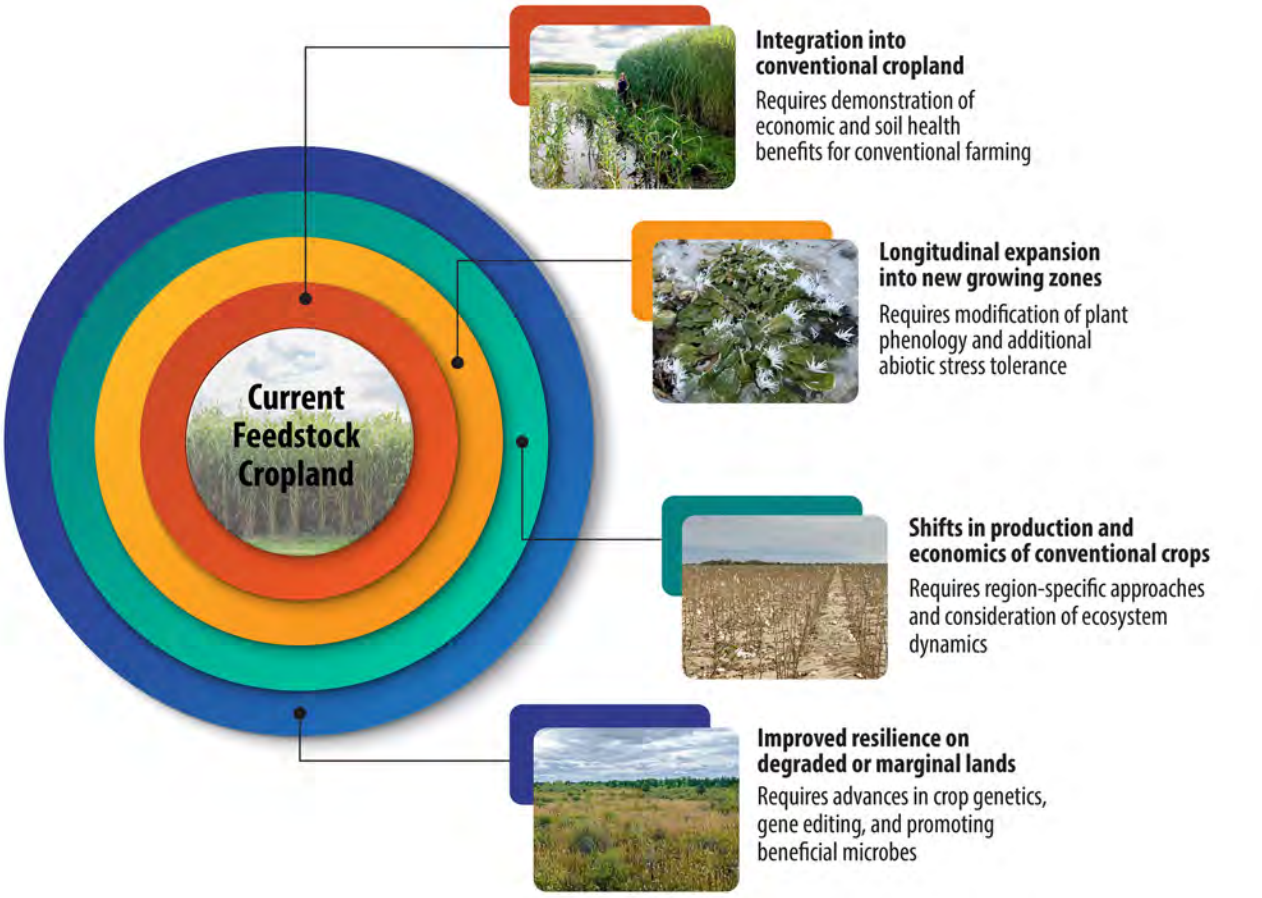

Fig. 1.1. Feedstock Crop Expansion Opportunities. Emerging and strategic opportunities for nonfood feedstock crops can expand and enhance existing feedstock production over space, time, and markets for energy, chemicals, and bioproducts. These opportunities complement conventional crop production, take advantage of shifts in conventional crop production, or utilize lands not currently in production or with marginal profitability for conventional crop production. [Clockwise from top: Miscanthus, courtesy University of Illinois Urbana-Champaign. Pennycress, courtesy Illinois State University. Cotton, courtesy Adobe Stock. Natural meadow, courtesy Adobe Stock. Sorghum, courtesy Center for Advanced Bioenergy and Bioproducts Innovation]

(Sage et al. 2015; Poudel et al. 2019). However, warming winters may reduce this concern and further expand the potential growing season for some feedstocks. Additional abiotic stress tolerance, including drought, heat, and salinity tolerance, will likely further enable range expansion for nonfood feedstock crops.

Reclaiming Low-Productivity Conventional Cropland

Shifts in the production and economics of conventional crops can create new opportunities for feedstock crop production. The productivity of agricultural land can decline for conventional crops due to decreased water availability, salinization, extreme weather events, and pathogen spread (Reeves and Liebig 2016; Jones et al. 2019; Thaler et al. 2021; Hultgren et al. 2025). Such lands may provide production opportunities for feedstock crops that are specifically bred or managed to thrive under challenging conditions.

Region-specific approaches and ecosystem dynamics considerations are necessary to optimize land use in response to long-term changes in environmental

conditions. For example, the irrigated area in the U.S. Great Plains will likely decline, thus impacting row crop production (Evett et al. 2020) and potentially opening opportunities to expand drought- and heat-tolerant sorghum and switchgrass production. After a conventional crop fails, a rapid transition to drought-tolerant feedstock crop production can avoid long fallow periods with potential topsoil and soil organic matter losses—soil features that critically support crop resilience. Diseases that cause regional collapses of conventional crop production, such as citrus greening in Florida (Singerman 2024), are creating opportunities for feedstock crops to thrive on previously productive cropland. Additionally, the decline of tobacco farming (Evett et al. 2020) and pine forests (Ojha et al. 2021) opens opportunities for feedstock crop production in the Southeast.

Improving Crop Resilience on Marginal Lands

Advances in feedstock crop genetics and gene editing can improve the suitability of crops for growth on marginal lands and thus expand the amount of land available for crop production. Understanding the genetic basis for specific resilience traits, such as salt or flood tolerance, can inform strategies to engineer and breed productive feedstock crop genotypes on degraded or marginal lands (Chen et al. 2017; Yu et al. 2023). Technological advances are enabling scientists to interrogate and domesticate new crop species that can phytomine critical minerals on underutilized marginal lands (Nkrumah et al. 2016; Reeves et al. 2018; Kikis et al. 2024). These species, including *Odontarrhena chalcidica* (previously known as *Alyssum murale* or *Alyssum chalcidicum*), *Odontarrhena corsica* (previously known as *Alyssum corsicum*), *Streptanthus polygaloides*, and *Dicranopteris pedata*, may enhance the overall suitability of land for crop production. Feedstock crops can also remediate soil from acidification and heavy metal contamination (Li et al. 2011), further increasing arable land acreage.

While locally adapted traits are needed to expand feedstock crops into new regions, potentially invasive crop traits should also be considered. For example, concern exists that some varieties of *Miscanthus sinensis* or hybrid miscanthus produce seeds that could escape field sites (Quinn et al. 2010; Bonin et al. 2017) or negatively impact soil biodiversity (Brami et al. 2020). In contrast, wild pennycress is naturally a nuisance weed but introduced domestication traits have largely eliminated its weediness (Gautam et al. 2026). Alternatively, adapting existing feedstock crops to new conditions reduces the risk associated with introducing new, potentially invasive species. Crop diversity enables productivity in regionally diverse environmental conditions, so adapting both current and emerging feedstock crops is warranted.

The following chapters explore key knowledge gaps related to plants (see Ch. 2, p. 7) and plant–microbe interactions (see Ch. 3, p. 31) that must be addressed to overcome environmental challenges to resilient feedstock crop production. Also discussed are research strategies and opportunities to make advances in these critical areas. Ch. 4 (p. 45) addresses additional research opportunities in expanding and designing resilient cropping systems. Ch. 5 (p. 49) describes technological innovations, shared resources and infrastructure, and workforce developments needed to enable and accelerate breakthroughs in feedstock crop resilience research.

Chapter 2

Improving Crop Resilience and Productivity

Plant productivity is highly dependent on resource availability and the dynamic interplay of environmental factors over a plant's lifespan. Suboptimal availability of one or more resources, either concurrently or sequentially, limits productivity in many agricultural systems. Adverse environmental conditions, including extremes in water availability (i.e., drought, waterlogging, and flooding), cold and heat stress, and low soil fertility, are major abiotic stressors that commonly limit productivity. Additionally, high soil salinity, pH extremes, and other chemical and physical soil characteristics, while generally more localized, impose significant constraints on plant growth and yield.

Productivity can be curtailed acutely or chronically, depending on the type of environmental stressor. Further, the timing, duration, and severity of stressors, especially with respect to plant developmental stage, critically influence stressor impact on productivity and survival. Different species, as well as genotypes within species, vary in sensitivity to specific environmental stressors. Thus, efforts to improve plant productivity and resilience in agroecosystems not only require optimal matching of plant species to production environment but also adapting genotypes to environmental conditions. To this end, a thorough understanding of the fundamental mechanisms underlying plant responses and adaptation to adverse environmental conditions is imperative.

Workshop discussions explored knowledge gaps in improving plant resilience and productivity, including how single and multiple abiotic stressors impact plant form and function at different developmental stages and across organizational scales. Research opportunities were identified and organized by broad plant group (perennial grasses, trees, and annual crops) and primary abiotic stressors (i.e., heat, cold, drought, and nutrient availability). Discussions touched on other plants with potential as resilient nonfood feedstock crops, such as Crassulacean acid metabolism (CAM) plants that exhibit high biomass potential on arid lands considered marginal for conventional crop production (see sidebar, CAM Plants as Potential Feedstock Crops, p. 8). Other stressors, including waterlogging and flooding, salinity, soil pH, and impacts of the microbiome, were discussed to a limited extent with respect to interactions with other stressors and impacts on plant phenology. Increased resilience to stressors will enhance crop productivity and can expand the geographic range of production species.

Plant resilience and productivity can be improved by deepening the mechanistic understanding of how single and multiple abiotic stressors impact plant form and function at different developmental stages and across organizational scales.

The extent of understanding of abiotic stress response mechanisms differs considerably by stressor type and

Continued on p. 9

CAM Plants as Potential Feedstock Crops

Crassulacean acid metabolism (CAM) plants hold promise as feedstock crops in semi-arid regions and marginal or abandoned agricultural lands due to their intrinsic resilience to hot and dry conditions. CAM plants are generally three to six times more water-use efficient than plants with C_4 and C_3 photosynthesis, respectively, and thus exhibit much lower water requirements annually than conventional feedstock crops (Borland et al. 2009; Yang et al. 2015).

Such high water-use efficiency (WUE) is largely due to the ability of CAM plants to open their stomata (microscopic leaf pores) at night to engage in nocturnal carbon assimilation and storage of malic acid when conditions are cooler and more humid than during the day, thereby limiting transpirational water loss (Winter and Smith 2021; Sage et al. 2023). The accumulated malic acid is then decarboxylated and refixed during the daytime with stomata closed. These unique features of CAM plants require distinct circadian, metabolic, and physiological regulatory processes (Winter and Smith 2021; Lim et al. 2025). An added benefit of partial or full daytime stomatal closure is elevated carbon dioxide concentrations within the plant, which increases photosynthetic efficiency.

CAM plants are globally widespread (Holtum 2023) and taxonomically distributed throughout vascular plants, occurring in 38 plant families and an estimated 370 genera. This diversity helps them adapt to specific ecological and agroecosystem niches with limited water availability (Gilman et al. 2023). CAM plants also display tremendous diversity in the degree to which they engage in CAM. This is modulated by developmental processes, anatomy, and environmental stressors and results in a range of facultative and obligate phenotypes adapted to prevailing water-limited conditions for survival or reproduction (Winter 2019; Messerschmid et al. 2021).

Among CAM plants, *Agave* and *Opuntia* species are emerging feedstocks for bioenergy and bioproduct production due to their high biomass potential (see figure, Plants Using Crassulacean Acid Metabolism Hold Promise as Bioenergy Crops in Semi-Arid and Marginal Lands, p. 9; Yang et al. 2015; Owen et al. 2016; Davis et al. 2019; Honorato-Salazer et al. 2021; Carvajal et al. 2025). Field trials for *Agave* and *Opuntia* species have demonstrated their potential as viable domestic feedstock crops in the United States (Davis et al. 2015; Neupane et al. 2021, 2024), with *Agave* species primarily used in bioethanol production (Yan et al. 2011) and *Opuntia* species used to produce both bioethanol (Santos et al. 2016; Latifa and Ettayebi 2021) and biogas (Mason et al. 2015; Krümpel et al. 2020; Lueangwattanapong et al. 2020). Both feedstocks provide advantages related to fossil energy inputs, trace gas emissions, water requirements, and use of arid lands ill-suited for food crops (Davis et al. 2014, 2019).

Genomic resources, including whole-genome sequences, are now available for more than 20 CAM species (Gilman et al. 2023) in genera including *Agave* (Yang et al. 2023), *Opuntia* (Fawcett et al. 2025), and *Ananas comosus* (pineapple; Ming et al. 2015; Chen, L.-Y., et al. 2019). These resources will inform biodesign efforts to introduce engineered versions of CAM into C_3 and C_4 crops to improve their WUE and drought tolerance (Borland et al. 2014; Lim et al. 2019; Schiller and Bräutigam 2021; Perron et al. 2024; Yang et al. 2024; Wu, S., et al. 2025).

To successfully design and engineer a fully functional CAM cycle into non-CAM plants will require a more comprehensive understanding of the enzymatic, transport, anatomical, regulatory, and signaling components and modules of CAM than

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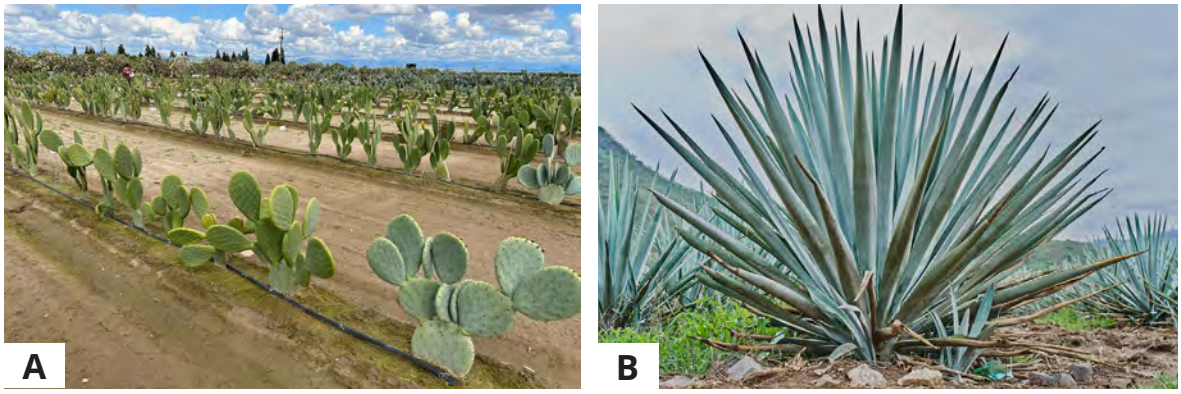
is currently available (Lim et al. 2025). Engineering co-adaptive traits of CAM plants into C_3 and C_4 photosynthetic species (Niechayev et al. 2019), such as tissue succulence (Lim et al. 2020) or reduced stomatal densities (Gray and Dunn 2024), is also an important strategy for developing resilient feedstock crops. CAM plants with high productivity, such as perennial *Agave* and *Opuntia* species, provide excellent opportunities to expand resilient bioenergy and bioproduct crop production under warm and dry conditions typically associated with marginal, degraded, or abandoned agricultural lands without displacing conventional crop production. Furthermore, introducing the CAM cycle or CAM-related traits (e.g., tissue succulence, reduced stomatal densities, and increased cuticular barriers to water loss) present beneficial strategies to improve the environmental resilience of other bioenergy and bioproduct crops in the future.



Plants Using Crassulacean Acid Metabolism (CAM) Hold Promise as Bioenergy Crops in Semi-Arid and Marginal Lands. CAM plants, such as species of *Opuntia* **(A)** and *Agave* **(B,** *A. tequilana* shown here), thrive in such environments because of their ability to take in carbon dioxide at night and re-assimilate it during the day with their stomata (i.e., leaf pores) closed, thereby reducing water loss from the pores during the heat of the day and conserving scarce water resources. [(A) Reprinted with permission from John Wiley and Sons from the *Journal of Agronomy and Crop Science* March 2024 cover image. See also Neupane, D., et al. 2024. "Biomass Production of 14 Accessions of Cactus Pear (*Opuntia* spp.) Under Semi-Arid Land Conditions," *Journal of Agronomy and Crop Science* **210**(2), e12705. DOI:10.1111/jac.12705. (B) Courtesy Adobe Stock.]

Continued from p. 7

feedstock crop species. As such, specific sections in this chapter are dedicated to primary abiotic stressors (see Section 2.2: Knowledge Gaps About Primary Abiotic Stressors Affecting All Plant Groups, p. 13) and plant groups (see Section 2.3: Strategies and Opportunities To Improve Perennial Grasses, p. 15; Section 2.4: Strategies and Opportunities To Improve Trees, p. 21; Section 2.5: Strategies and Opportunities To Improve Annual Crops, p. 25). However, workshop discussions also revealed several overarching aspects that apply to all plant groups. These are outlined in the first section of this chapter.

2.1 Knowledge Gaps in Factors Impacting Stress Responses Across Plant Groups

Under field conditions, plants are exposed to complex, increasingly variable, and often extreme environments that threaten their growth and survival. Although

adverse environmental conditions affect species in distinct ways, several shared challenges must be addressed to improve resilience of diverse feedstock crops. This section outlines key knowledge gaps shared among perennial grasses, trees, and annual crops and emphasizes the importance of ensuring that research findings are applicable to real-world conditions. Central gaps include understanding how individual and combined abiotic stressors impact plant form and function across developmental stages and biological scales of organization, as well as how the timing, duration, and severity of these stressors shape overall plant performance.

Understanding the hierarchy of plant stress response processes and their interconnectedness across different levels of biological organization is critical for designing more resilient feedstock crops.

Integration Across Biological Scales of Organization

Tremendous knowledge gaps remain for determining how genetic and environmental factors combine to influence and link fundamental plant processes (e.g., photosynthesis and respiration) to productivity. Progress in improving plant stress tolerance hinges on a thorough understanding of the interconnectedness and hierarchy of plant stress responses across all levels of biological organization. Stressor impacts are a function of organizational scale as different cells, tissues, and organs can exhibit varied responses to given stressor characteristics. Consequently, a critical research priority is to develop a better understanding of plant stress responses across plant organizational scales (from the subcellular to the canopy) and cropping system levels (see Fig. 2.1, p. 11). This includes dissecting genetic, molecular, and regulatory mechanisms underlying individual physiological traits, such as stomatal behavior and carbon allocation under stress, and determining how temporal and developmental aspects modulate stress resilience.

Designing more stress-resilient plants and cropping systems requires collaborations among investigators with complementary expertise interrogating stress response processes at different plant organizational scales and the interconnectedness of processes and trade-offs across levels of biological organization (Passioura 2010). In addition to gaining a better understanding of the hierarchy of stress responses across organizational scales, researchers must address the relatively limited understanding of belowground processes with root-focused research. Although root systems are central to plant adaptation across all major stressor types, as well as for plant–microbe interactions, they remain woefully understudied. Rather than investigating roots and root systems across organizational scales in isolation, research should consider organism-level dynamics, such as source–sink relationships and root–shoot and shoot–root signaling. However, studying roots, especially under field conditions, is technically challenging (Mehra et al. 2025). Thus, a greater focus on root systems should be accompanied by developing novel, innovative approaches to accelerate research progress and ultimately translate findings into more stress-resilient crops (see Ch. 5: Enabling Technologies, Shared Resources, and Infrastructure Needed for Resilient Crop Production, p. 49).

Despite significant advances in genetics and molecular biology, understanding of the genes, regulatory networks, and genetic and epigenetic mechanisms that control many plant abiotic stress responses remains incomplete. To unravel the complexities of plant stress responses, researchers are increasingly turning to multiomics technologies such as single-cell RNA sequencing, ATAC (assay for transposase-accessible chromatin) sequencing, spatially resolved proteomics and metabolomics, and AI and machine learning (AI/ML) tools and methodologies. While such studies are expected to provide fundamental insights into stress response mechanisms, they must be accompanied by thorough characterization of physiological processes that elucidate stress response hierarchies along the molecular to organismal scales using approaches relevant to field conditions. The impact of these investigations can be further enhanced by standardizing basic measurement

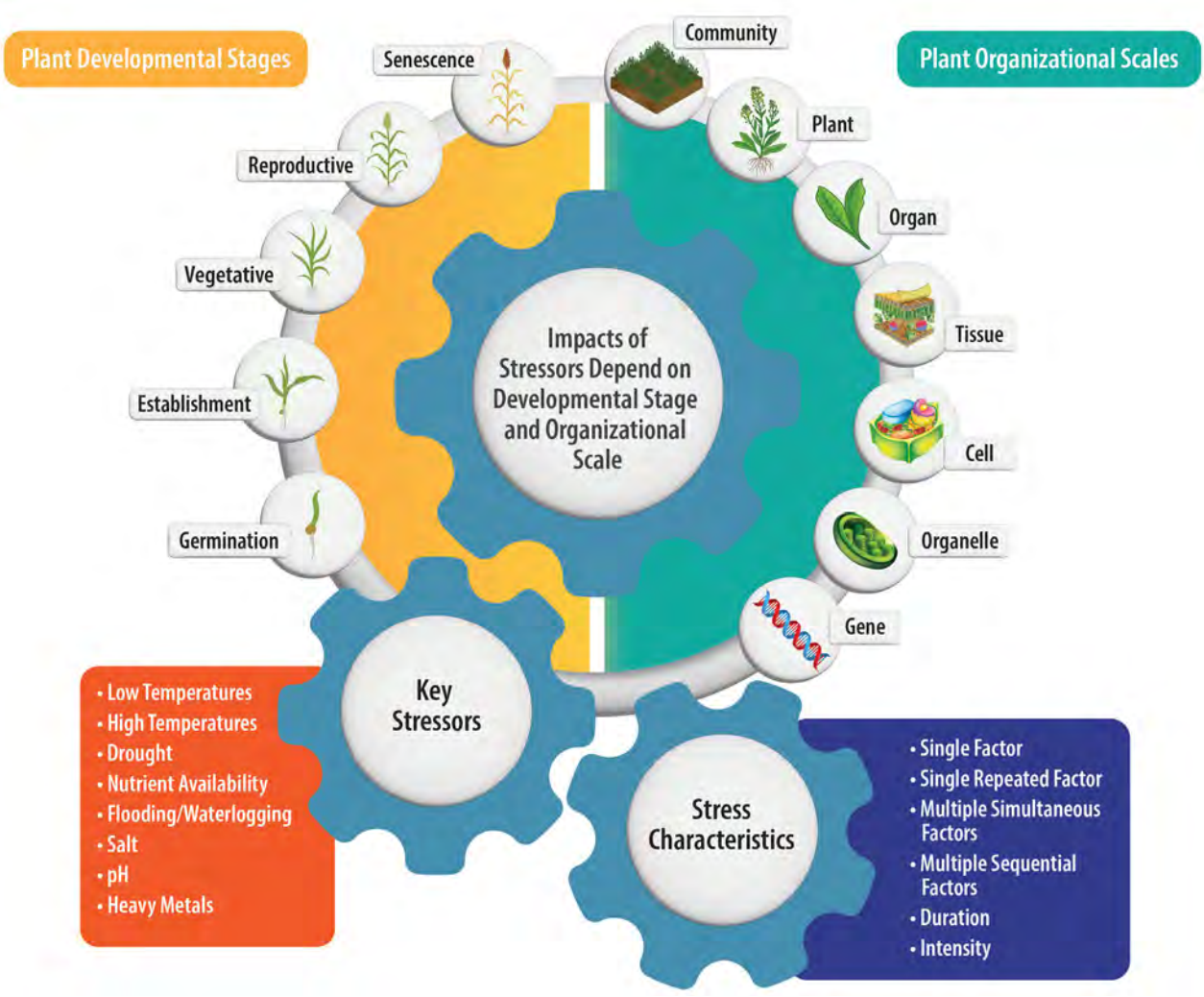


Fig. 2.1. The Impacts of Key Environmental Stressors on Plant Performance Are Strongly Influenced by Stress Characteristics and Timing Relative to Plant Developmental Stage. Understanding the effects of plant stress responses on every organizational scale and the hierarchy of responses across organizational scales is critical to improving stress resilience and performance of crops under field conditions.

procedures and collecting comprehensive metadata, thus facilitating comparative analyses and providing strong foundations for modeling efforts that bridge scales of organization. Leveraging AI/ML approaches to, for example, extract meaningful patterns from the data, prioritize candidate genes, and scale predictive modeling (see Ch. 5: Enabling Technologies, Shared Resources, and Infrastructure Needed for Resilient Crop Production, p. 49), will improve understanding of the interrelatedness and hierarchies of stress response processes across organizational scales (Li et al. 2025; Singhal et al. 2025).

Timing, Duration, and Severity of Stress

The impacts of stressors often depend heavily on a combination of timing and severity relative to plant developmental stage (Wang, D., et al. 2016; Oguz et al. 2022). Impacts are also strongly influenced by characteristics like duration (see Fig. 2.1, this page),

with short-term acute (i.e., extreme) stress events impacting plants differently than chronic stress. For example, an insufficient period of cold temperatures (failed vernalization) can prevent flowering, while too much heat during pollen development can impair pollen viability and consequently reproductive success (Amasino 2010; De Storme and Geelen 2014).

Because stressor impacts are also a function of organizational scale, it is necessary to acknowledge the dynamic nature of short-term physiological stress responses, the ability of plants to physiologically acclimate to stress over longer timescales, and the impact of biochemical or developmental feedbacks within or across growing seasons. As such, dissecting stress responses across broad spatiotemporal resolutions is critical for effectively leveraging tolerance mechanisms to improve stress resilience.

Translating Relevant Research to Field Conditions

Considerable progress has been made in dissecting plant stress responses in controlled environments, but a key challenge remains in translating findings from the laboratory to the field (Deikman et al. 2012; Purugganan and Jackson 2021). Controlled environment studies yield important mechanistic data but often fail to replicate the complexity of field conditions (Plessis 2023). Indeed, many phenotypes observed in controlled environments do not translate to the field or, conversely, they may only manifest under field conditions, presenting a disconnect that highlights the importance of conducting field experiments (Passioura 2020). Similarly, testing commercially relevant varieties and modified germplasm in the field is critical because basic research on noncommercial varieties may not translate well to a commercial production setting.

Scaling up from controlled environments to field- and landscape-level studies is a high-priority need that can enable researchers to assess how stress-response traits translate across environments and predict resilience under future environmental conditions. Integrated studies conducted in multiple, diverse field environments are needed to elucidate the links between molecular mechanisms, physiological processes, and

Plant stress resilience research should focus on field-relevant stress conditions and involve multi-environment field experiments coupled with intensive phenotyping and environmental sensing.

plant performance in complex field conditions across climatic zones (Napier et al. 2023). Field experiments that are well monitored with environmental probes (e.g., to measure above- and belowground temperature and moisture) and drone and satellite imagery are critical. Field analyses must be conducted throughout the growing season to capture stress events, diurnal cycles, effects from a changing rhizosphere, and developmental stages. Newer spatially resolved molecular approaches, such as single-cell omics, need to be coupled with assessments of physiological trait expression and plant performance under field conditions. Multiseason, multisite trials and long-term studies that capture the spectrum of environmental variation can be coupled with AI/ML-assisted modeling approaches to help translate mechanistic insights from laboratory-based studies into field-applicable strategies.

To acquire meaningful datasets in a reliable manner, researchers must be able to impose stress conditions in the field (e.g., by employing rainout shelters, water logging, fertilizer regimes, soil types, and rhizosphere conditions). Long-term common garden experiments located in different climatic regions are essential to determining relative responses and performance of genetically diverse germplasm in different environments, understanding the genetics underpinning beneficial traits, and enabling pre-breeding efforts. Relative yield performance is a key indicator of a plant's ability to handle environmental perturbations and chronic stress.

Given the complexity and generally polygenic nature of plant stress responses, molecular and physiological approaches should be leveraged to characterize natural variation, and quantitative genetic analyses should be used to identify functional alleles and

genes. These endeavors enable genomic prediction to accelerate development of feedstock species with improved stress resilience. To this end, a key priority for the research community is to continue developing and protecting shared germplasm resources (see Ch. 5: Enabling Technologies, Shared Resources, and Infrastructure Needed for Resilient Crop Production, p. 49). Critical to leveraging these resources are advances in diverse, high-throughput phenotyping technologies and their applications in multiple well-monitored (i.e., with environmental probes), real-world environments. Such advances can empower and integrate quantitative genetic approaches into multiomics approaches with the goal of achieving, for example, efficient identification of candidate genes, targeted genetic modifications, and accelerated adaptive introgression of beneficial traits into elite germplasm.

Combined Stressors

Under field conditions, plants often experience multiple stressors that may occur simultaneously, overlap partially, occur sequentially, and vary in relative severity (Mittler 2006; Lamaoui et al. 2018). For instance, drought and heat stress often co-occur, whereas waterlogged conditions may occur before drought conditions or vice versa. Resilience to diverse multifactorial stressor combinations requires plant responses coordinated by fine-tuned mechanisms that also account for aspects of plant development (Zandalinas et al. 2021). Most studies of plant stress responses have examined single stressors and ignored the complexities of interacting stressors inherent in field conditions. The limited number of studies on multifactorial stressor combinations conducted to date reveal varied physiological and complex molecular responses, including responses unique to specific combinations (Bulut et al. 2025). Understanding these responses in feedstock species requires integrative approaches that consider both timing and intensity of stress imposition, especially as environmental variability increases.

Studying different scenarios of realistic stressor interactions is challenging, particularly under field conditions, and the number of possible stressor combinations can quickly become unmanageable. Suitable experimental facilities and field sites that enable testing of realistic stressor combinations are needed; these could serve as core locations that host highly collaborative, interdisciplinary research projects. Although challenging, accounting for the complexities of stressor combinations is critical to developing breeding and engineering targets for enhanced plant resilience.

2.2 Knowledge Gaps About Primary Abiotic Stressors Affecting All Plant Groups

Abiotic stressors relevant to all plant groups include low and high temperatures, drought, and nutrient availability. Priorities in plant abiotic stress research can be identified by answering key questions for target species, including: Which abiotic stressors impose the greatest yield losses? Which plant traits or processes are most vulnerable and at which developmental stages? Which plant traits can be engineered to increase resilience to these stressors?

Low Temperatures

Understanding how plants respond to cold at various developmental stages is imperative to improving winter survival and productivity. Vernalization—the process in which plants require an extended period of cold temperatures before flowering in the spring—and photoperiod are critical developmental signals that condition vegetative and reproductive growth, and thus impact adaptation to different production regions. Temperature fluctuations can interfere with acclimation processes that normally prime plants for low temperatures, leading to reduced vigor and survival, particularly in winter annuals and perennials. Key aspects of plant development and physiology that require improved understanding to increase feedstock species resilience to low temperatures include:

- How circadian, seasonal, and developmental cues and their interactions influence low-temperature responses.
- Which mechanisms control vernalization and the transition to reproductive growth when plants are less stress tolerant.

- Which mechanisms control winter dormancy and survival.
- Which mechanisms underlie acclimation to low temperatures.
- Which traits condition recovery from low temperatures.
- How epigenetic mechanisms affect plant adaptation to low temperatures.
- How low temperatures interact with other abiotic stress factors, particularly low soil fertility, ice and snow cover, and light intensity.

High Temperatures

Temperature fluctuations, including heat waves and chronic high temperatures, influence plants throughout their life cycles, affecting critical developmental transitions and reproductive processes (Jagadish et al. 2021; Bakery et al. 2024; Distéfano et al. 2025). Many crops are particularly sensitive to high temperatures during flowering, fertilization, and seed set when disruptions can drastically reduce reproductive success and yield (Qian et al. 2025). Understanding which growth phases are most vulnerable to elevated temperatures is essential to developing strategies that improve heat resilience.

The severity and duration of high-temperature stress also impact crop productivity. Daily maximum temperatures and high nighttime temperatures can both impose significant stress. Stress interactions (see Combined Stressors, p. 13) are particularly relevant to heat stress because heat stress often coincides with limited water availability. Such interactions magnify the complexity of plant stress responses and compound impacts on plant productivity (Cohen et al. 2020). A better understanding of diverse aspects of plant tolerance to high temperatures is needed to enhance resilience, including the impacts of high temperatures on:

- Critical developmental transitions in plants, with an emphasis on the timing and phenological stage (e.g., vegetative growth, flowering, fertilization, and seed set) during which stress occurs.
- Physiological processes, including photosynthesis and respiration.
- Metabolic processes and bottlenecks, such as in structural polymer synthesis and enzyme activities.
- Epigenetic and legacy effects of past environmental conditions, especially in perennial plants.
- The complex interplay of environmental variables, such as stress duration, intensity, and daily temperature fluctuations (i.e., day versus nighttime heat); wet versus dry heat; and heat and drought interactions.

Drought

Plants in many environments experience some level of water deficit stress at one or more developmental stages. Both the limited availability of soil water and a high vapor pressure deficit (i.e., atmospheric drought) can contribute to drought stress (Grossiord et al. 2020). As with heat stress, the timing of drought stress relative to plant developmental stage plays a crucial role in determining its impact. Similarly, drought seldom occurs in isolation, and its effects are magnified by interactions with other stressors (Sinha et al. 2021). Plant responses to drought stress have been well studied, but many aspects remain unclear, particularly in feedstock species, including:

- Mechanisms controlling root and shoot growth plasticity and source–sink dynamics.
- Stomatal traits and leaf surface characteristics that influence trade-offs between water loss and carbon dioxide uptake.
- The interplay between xylary and extra-xylary components of the plant hydraulic pathway.
- Osmoregulation and nutrient uptake and allocation.
- Spatiotemporal pattern of root growth and function.
- Root exudate quantities and composition under drought and their effects on the microbiome.

- Stress interactions, particularly the simultaneous occurrence of drought and heat stress, as well as the sequential occurrence of drought stress before or after waterlogged conditions.
- Acclimation processes; transgenerational mechanisms; and, in perennial species, long-term legacy effects of drought conditions.

Nutrient Availability

The mechanisms plants use to acquire, distribute, and remobilize nutrients are critical for their adaptation to different environments and to maximize productivity and financial profitability (Brant and Chen 2015). Many feedstock species are targeted for planting on marginal lands, so their nutrient acquisition and use efficiencies are particularly important to achieving high productivity. Root characteristics, including interactions with the microbiome, are key to nutrient acquisition (Wang et al. 2024). Playing a key role in nutrient-use efficiency are processes like nutrient remobilization—the movement of nutrients within the plant among vegetative tissues and eventually from shoot to seeds or roots at the end of the growing season. Nutritional status and use efficiency may also influence feedstock composition (e.g., nitrogen availability affects seed oil and protein concentrations). To optimize nutrient acquisition and nutrient-use efficiencies in feedstock species, the following knowledge gaps should be addressed:

- Mechanisms and control of nutrient uptake across plant root system organizational scales, across temporal scales, and through microbiome interactions.
- Mechanisms and control of long-distance nutrient transport and allocation within plants, including belowground versus aboveground responses to nutrient availability; nutrient mobilization from shoot to seeds, roots, or rhizomes; and understanding genetic and physiological mechanisms controlling plant carbon and nitrogen balance (e.g., how photosynthesis and nitrogen assimilation interact to control nitrogen-use efficiency (NUE).
- Mechanisms mediating the effects of nutrient availability on tissue and seed composition.
- Interactions between nutrient uptake, allocation, and use efficiencies with other stressors, especially water availability and water-use efficiency (WUE).
- Mechanisms and control of nitrogen retention in soil, including plant regulation of diverse microbe-mediated nitrogen loss pathways (e.g., nitrification, denitrification, anaerobic ammonium oxidation, and volatilization).

Other Abiotic Stressors

Workshop participants discussed other abiotic stressors, including flooding and waterlogging, salt, heavy metals, soil pH, and soil physical strength, to a limited extent but recognized critical needs for additional research in these areas. Although each stressor is individually important primarily on local and regional scales, workshop participants largely discussed their impacts as part of stressor combinations. Combinations of abiotic stressors that need more research attention include salt and drought stress; the impacts of pH on heavy metal toxicity and nutrient availability; and the interactions of various stressors with flooding, waterlogging, and high and low temperatures.

2.3 Strategies and Opportunities To Improve Perennial Grasses

Perennial grasses that carry out C_4 photosynthesis have long been championed as feedstocks for bioenergy and bioproducts due to their many advantageous physiological and agronomic attributes. These species are some of the most prolific plants in the world in terms of biomass productivity due to their efficient C_4 photosynthesis, which gives them a major advantage in hot, dry, and high light environments. As perennials, these grasses also have deep root systems that form soil organic matter, contributing to long-term resilience and soil health.

Switchgrass (*Panicum virgatum*), miscanthus, sugarcane and energy cane (*Saccharum* species hybrids), and other C_4 perennial bioenergy grasses are ideal

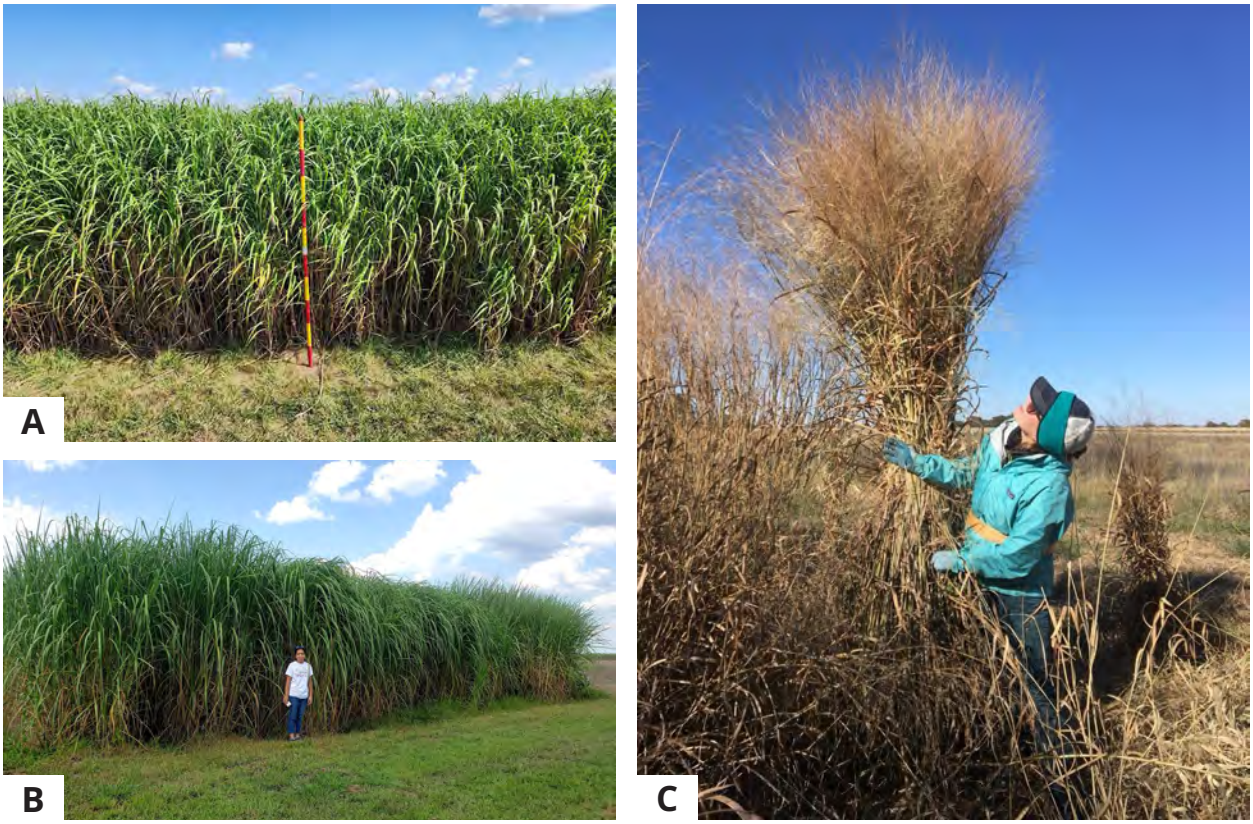


Fig. 2.2. Perennial C_4 Bioenergy Grasses Display High Aboveground Biomass Production. (A) A seven-year-old stand of *Miscanthus x giganteus* at the Iowa State University Sustainable Advanced Bioeconomy Research farm in Ames, Iowa. **(B)** Sugarcane grown at the U.S. Department of Agriculture (USDA)–Agricultural Research Service's Sugarcane Research Unit field site in Houma, La. Genetic diversity among the sugarcane cultivars is evident from phenotypic variation in leaf architecture. **(C)** Switchgrass plant at the end of its third growing season near Columbia, Mo. The plant is tied up and ready for harvest and biomass determination. [(A) Courtesy Iowa State University, (B) Courtesy USDA–Agricultural Research Service's Sugarcane Research Unit, (C) Courtesy University of Missouri]

candidates for U.S. bioenergy production, demonstrating widespread productivity and yield stability in field trials (see Fig. 2.2, this page). Numerous other perennial grass species also show promise as bioenergy crops (see sidebar, Additional Perennial Grass Species with Potential as Bioenergy Crops, p. 17).

Despite the advantageous traits of perennial bioenergy grasses, more research is needed to determine how genetic and environmental stressors combine to influence fundamental processes regulating these plants' physiology, productivity, and long-term stand persistence. This section discusses opportunities to build and maintain research and data resources—such as germplasm stocks, genetic tools, field sites, and data repositories—to better understand how bioenergy grasses allocate resources (e.g., carbon and nutrients) where they are most needed to enhance productivity and resilience.

Building and Maintaining Resources for Mechanistic Studies and Genetic Improvement

The vast majority of efforts to improve bioenergy grasses have employed traditional breeding and cultivar

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Additional Perennial Grass Species with Potential as Bioenergy Crops

In addition to well-characterized switchgrass, miscanthus, and *Saccharum* bioenergy crops, several other perennial grass species warrant consideration and further study. These include C_4 perennial grasses such as Napier grass (*Cenchrus purpureus*), big bluestem (*Andropogon gerardi*), and prairie cordgrass (*Spartina pectinata*). These species may be especially suited to expanding bioenergy feedstock growing regions and mitigating certain risks of low productivity associated with extreme environments or disturbances.

Napier Grass (*Cenchrus purpureus*)

Napier grass (also known as elephant grass) is native to tropical and subtropical regions of Africa where it occurs along rivers, floodplains, and disturbed mesic areas. It is a remarkably productive grass in subtropical and tropical climates (see figure panel A, Seeking Alternative Bioenergy Crop Species, p. 18), is salt tolerant, and recovers quickly from coastal disturbances like tropical storms and hurricanes, salt intrusion, and lodging. Napier grass may be suited to the U.S. Gulf Coastal Plain region (Chiluwal et al. 2019; Johannes et al. 2024), but its value as a feedstock will require advances in controlling its invasiveness. One possible solution is to hybridize Napier grass with pearl millet (*Pennisetum glaucum*), generating sterile triple hybrids often called King grass.

Big Bluestem (*Andropogon gerardi*)

In contrast to Napier grass, big bluestem is a native C_4 perennial grass with a widespread distribution across North America. It is a dominant grass in most prairie habitats ranging from eastern tallgrass prairie to drier regions of the Southern Great Plains. Historically, it contributed as much as 80% of Midwest tallgrass prairie biomass. Like switchgrass, big bluestem is long-lived with regionally adapted ecotypes (see figure panel B, p. 18; McMillan 1959; Galliart et al. 2018). Its deep roots and drought resilience make it suitable for production on marginal and degraded lands. Big bluestem could be a viable feedstock for many of the same U.S. regions as switchgrass while also filling a niche in more arid regions. Although it has a long history of breeding for biomass yields, this species' available genetic variation provides opportunity for improvements in biomass yields and adaptation (Casler et al. 2018).

Prairie Cordgrass (*Spartina pectinata*)

Like big bluestem, prairie cordgrass is a native C_4 perennial with a wide distribution across most of eastern North America. It naturally occurs in wet prairies and wetlands and is highly flood-tolerant (see figure panel C, p. 18). It could be a valuable feedstock addition in the Midwest and northern Great Plains, especially on marginal farmland prone to flooding or with poor drainage (Boe et al. 2009). The variation in performance among different prairie cordgrass populations at marginal sites (e.g., wet, high salinity, or low fertility) indicates ample opportunities for breeding for greater abiotic stress tolerance (Lee et al. 2024).

Each of these species has been evaluated from an ecological and agronomic perspective, and genetic and genomic resources for their study and improvement are expanding. Big bluestem and prairie cordgrass are especially promising feedstock candidates given their native status and broad distributions in the United States. One appealing idea is to consider planting mixtures of native perennial grasses (e.g., switchgrass, big bluestem, and prairie cordgrass) to leverage the potential benefits of genetic diversity and complementarity over large-scale monocultures (Hong et al. 2012; Lee et al. 2019).

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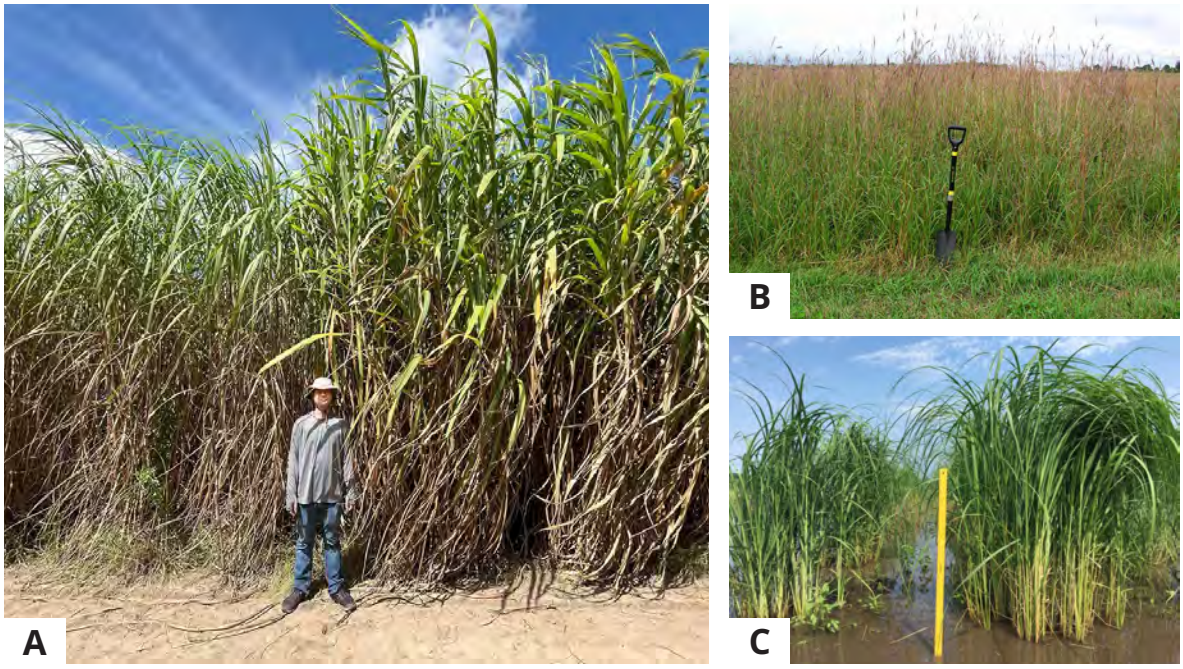
Like other perennial bioenergy grasses, these species have large complex genomes derived through hybridization and allopolyploidy. Advances in long-read DNA sequencing have enabled progress with published or draft genome assemblies for Napier grass and big bluestem (Yan et al. 2021; JGI 2025); further development is needed to leverage modern marker-based breeding and genetic engineering efforts for these and other perennial grasses.



Seeking Alternative Bioenergy Crop Species. Perennial grasses other than switchgrass, miscanthus, and *Saccharum* warrant further research as they may thrive in new regions or less favorable production environments. **(A)** Napier grass (right) was bred to achieve higher biomass yields than energy cane (left) when grown on marginal land with loamy sand soil in Citra, Fla. **(B)** A 12-year-old stand of "Sunnyview" big bluestem near Brookings, S.D., in late September. **(C)** Prairie cordgrass, shown here in Urbana, Ill., is tolerant of ponding, which can occur after intense rain events due to poor soil drainage. [(A) Courtesy University of Florida–Institute of Food and Agricultural Sciences; (B) Courtesy South Dakota State University; (C) Courtesy Department of Crop Sciences, University of Illinois at Urbana-Champaign]

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development, with a primary focus on forage and tissue quality, seed characteristics, and biomass yields using recurrent selection (Casler 2012; Clifton-Brown et al. 2019; Diniz et al. 2019). Field trials have centered on discovering regionally adapted plant material, and current agronomic practices depend on seeding or planting rhizomes of local ecotypes or cultivars that have undergone little to no genetic improvement.

More recently, the bioenergy grass research community has generated new resources, including genetic mapping populations (Milano et al. 2016; Dong et al. 2018), diversity panels, genome assemblies (Clark et al. 2014; Diniz et al. 2019; Mitros et al. 2020; Lovell et al. 2021; Healey et al. 2024), genetic engineering technologies (U.S. DOE 2024b), and transcriptomic data (Sreedasyam et al. 2023), to facilitate mechanistic studies and accelerate genetic improvement. These resources are critical for discovering key environmental

limits to stand productivity and persistence, as well as resources for developing big data to enable AI approaches for targeted improvement.

Critical steps the research community can take to advance understanding of fundamental mechanisms, leading to improvements in productivity and abiotic stress resilience of perennial bioenergy grasses, include:

- Creating new resources and protecting existing ones, including germplasm development and preservation, long-term field trials and common gardens established across a range of environments, and transformation and gene editing.
- Expanding the collection of in-field and aerial data and the use of existing and new enabling technologies, especially across temporal and spatial scales. Examples include crop phenotyping technologies (e.g., terrestrial laser scanning, unoccupied airborne vehicle-mounted lidar, and hyperspectral imaging spectroradiometer), and ground-penetrating radar (Yang et al. 2020).

The availability of genomic, germplasm, field, and phenotyping resources is critical to facilitating mechanistic studies and accelerating improvements in feedstock crop germplasm.

Understanding How Plants Allocate Resources To Enhance Performance, Stress Resilience, and Long-Term Persistence

A striking feature of most bioenergy grasses is their widespread natural distribution. Natural populations of switchgrass, for example, occur throughout much of eastern and central North America from Mexico to Canada, spanning almost 29 degrees of latitude (Lovell et al. 2021; Sacks et al. 2021; Zuloaga and Aliscioni 2023). Similarly, the natural ranges of *Miscanthus sacchariflorus* and *M. sinensis* span much of East Asia, from far eastern Russia and northeastern China through Southeast Asia (Anzoua et al. 2011; Ge et al. 2017). Sugarcane originated from hybridization among multiple *Saccharum* species in tropical Asia-Pacific regions (Okemo et al. 2024) and spread through much of Asia, the Middle East, and the Mediterranean region before being introduced in the New World (Pompidor et al. 2021). The ability of these grasses to persist across such large environmental gradients attests to their widespread adaptability, vigor, and stress hardiness and suggests they possess a host of possible adaptive traits to increase their performance and stress resilience across potential feedstock croplands (Heckman et al. 2024). Enhanced stress resilience (e.g., greater cold or salt tolerance) can also facilitate geographic range expansion and enable feedstock crop cultivation on previously unsuitable marginal lands (Hale et al. 2014; Sage et al. 2015; Tang et al. 2024).

Another distinctive aspect of perennial bioenergy grasses is their decades-long lifespan, which is enabled by their allocation of resources toward storage for future needs in addition to current growth and reproduction. Early in the growing season, bioenergy grasses rapidly develop their canopy and build biomass through photosynthesis. Later in the season, they move carbon and nutrients into underground rhizomes and roots to prepare for overwintering (see Fig. 2.3A, p. 20). Seasonal patterns of carbon and nutrient source–sink dynamics and modulations by environmental conditions can influence biomass production in a given year, can cause legacy effects that impact future production (see Fig. 2.3B, p. 20; Hawkes and Kiniry 2017), and have been associated with age-related productivity declines (Sharma et al. 2022; Tejera et al. 2022; Tejera-Nieves et al. 2023). Vegetative growth in bioenergy grasses largely stops once flowering begins, so delaying flowering can increase biomass yield (Jensen et al. 2013; Casler 2019). However, late-flowering plants risk losing nutrients if freezing occurs before nutrient remobilization can occur (Yang and Udvardi 2018; Tilhou et al. 2023). Freezing damage may deplete bud banks and, consequently, reduce plant tolerance to adverse conditions, limit plant responses

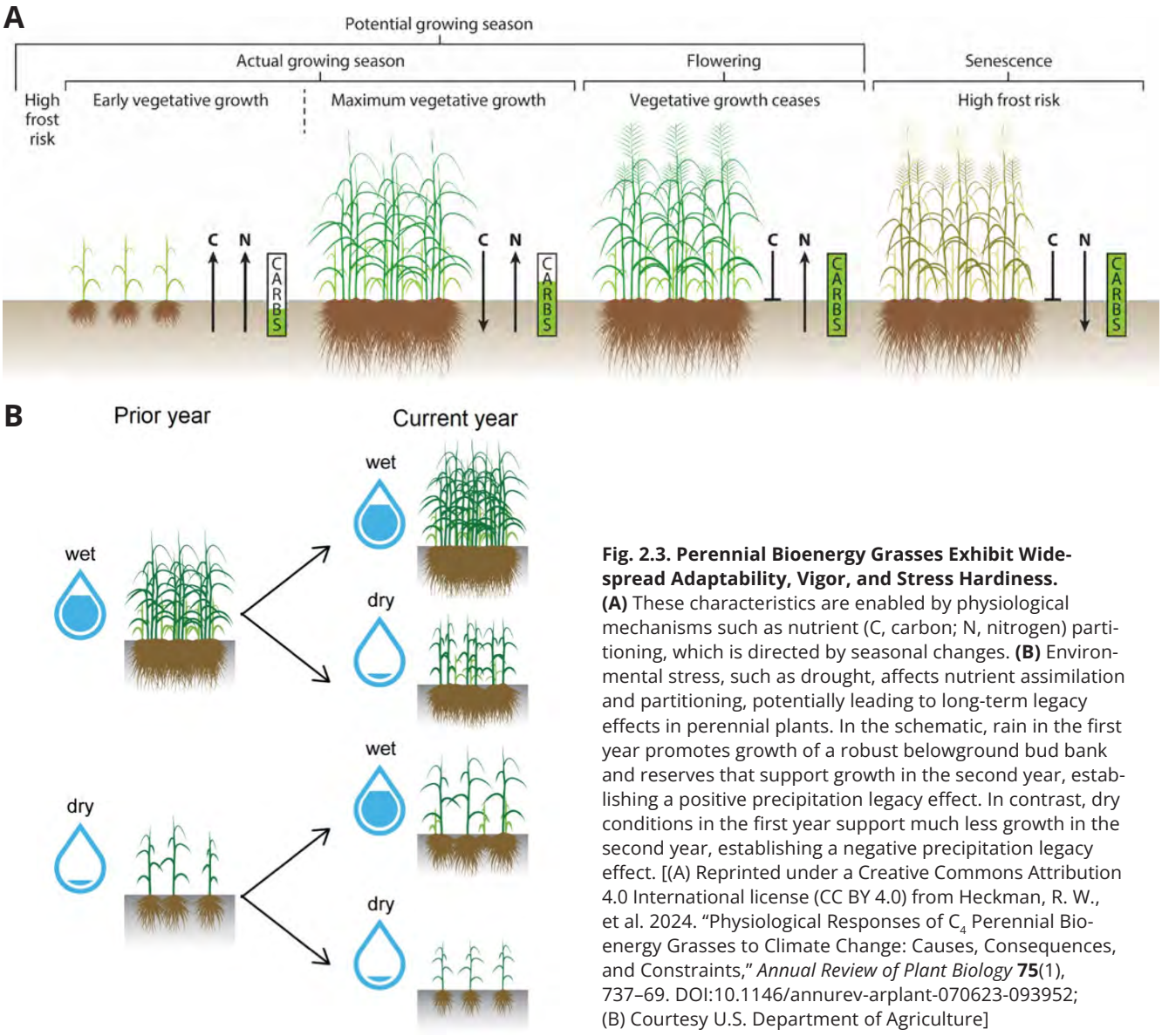

Fig. 2.3. Perennial Bioenergy Grasses Exhibit Widespread Adaptability, Vigor, and Stress Hardiness. **(A)** These characteristics are enabled by physiological mechanisms such as nutrient (C, carbon; N, nitrogen) partitioning, which is directed by seasonal changes. **(B)** Environmental stress, such as drought, affects nutrient assimilation and partitioning, potentially leading to long-term legacy effects in perennial plants. In the schematic, rain in the first year promotes growth of a robust belowground bud bank and reserves that support growth in the second year, establishing a positive precipitation legacy effect. In contrast, dry conditions in the first year support much less growth in the second year, establishing a negative precipitation legacy effect. [(A) Reprinted under a Creative Commons Attribution 4.0 International license (CC BY 4.0) from Heckman, R. W., et al. 2024. "Physiological Responses of C_4 Perennial Bioenergy Grasses to Climate Change: Causes, Consequences, and Constraints," *Annual Review of Plant Biology* **75**(1), 737–69. DOI:10.1146/annurev-arplant-070623-093952; (B) Courtesy U.S. Department of Agriculture]

to favorable conditions, and possibly produce legacy effects (see Fig. 2.3B, this page; Reichmann et al. 2013; Heckman et al. 2023).

Improved understanding of the molecular mechanisms underlying physiological processes can enable researchers to tap the genetic potential of perennial bioenergy grasses to significantly improve productivity, stress resilience, and long-term persistence. This strategy provides a direct route to enhanced plant performance through genetic modification and modern breeding. Processes, mechanisms, and aims of particular interest include:

- Increased photosynthesis and WUE.
- Acclimation of photosynthesis and respiration to temperature extremes and drought.
- Cold acclimation, freezing tolerance, and overwintering capacity.

- Regulation of growth and resource allocation across the growing season to improve performance in the short and long term. Specific targets include seasonal carbohydrate source–sink dynamics; carbohydrate storage in belowground rhizomes and bud banks; and mobilization of nitrogen, phosphorus, and other nutrients from senescing leaves to belowground tissues while accounting for potential trade-offs between productivity and persistence.
- Mechanisms of drought and temperature stress tolerance related to the accumulation of storage reserves; the quality and quantity of roots, rhizomes, and buds, including with respect to legacy effects; seedling establishment; and reproductive stages (e.g., seed production).
- Mechanisms underpinning invasiveness, especially of miscanthus.
- Microbiome–plant interactions in general and under specific stress conditions (see Ch. 3: Harnessing Plant–Microbe Interactions, p. 31).

2.4 Strategies and Opportunities To Improve Trees

Tree-based feedstock cropping systems must be capable of withstanding complex and often simultaneous stressors, including drought, flooding, pathogen attack, and extreme weather events, all while sustaining productivity over many years. As such, enhancing stress resilience is an important aspect of tree breeding efforts and may also expand the geographic range and marginal lands on which different species can be grown.

Abiotic stressors can leave legacy impacts on tree physiology and growth traits. The most immediate consequences involve changes in tree physiological traits (e.g., hydraulic conductivity) that can impact productivity over future growth seasons. Trees have also shown mechanisms of stress memory. For example, drought exposure can induce epigenetic modifications that persist over time and can prime the response to future stress (Kurpisz and Pawlowski 2022). In parallel, acute stress can reshape the tree microbiome. When drought modifies root microbial community composition, for instance, drought-tolerant tree genotypes develop a rhizosphere enriched with beneficial bacteria and mycorrhizal fungi (Kristy et al. 2022). Thus, trees retain a legacy of stress through epigenetic, microbial, and physiological changes, which together influence long-term productivity and resilience.

Better understanding of the physiological and molecular bases of seasonal and interannual growth, nutrient storage, and remobilization patterns will generate improvements in the performance, resilience, and long-term productivity of perennial bioenergy grasses.

Woody perennial species have been genetically improved for decades for timber, pulp, and paper production, with the primary goal of increasing biomass yield. These objectives closely align with the needs of tree cultivation for feedstock production. However, feedstock-focused forestry will require further development of existing germplasm from native species—such as those in the genus *Populus*—to maintain high productivity under environmental stress. In parallel, initial trials with non-native species, including those in the genus *Eucalyptus*, could be expanded to evaluate their adaptability and performance under local growing conditions. These efforts can be accelerated by better understanding the plant regulatory networks implicated in stress responses and translating results from these studies to field conditions.

Developing Candidate Tree Species for Feedstock Production

Several woody tree species have demonstrated suitability as bioenergy feedstocks in pilot-scale and localized commercial applications, including those in the genera *Populus*, *Salix*, *Pinus*, and *Eucalyptus* (see Fig. 2.4, p. 22). The species are characterized by very rapid

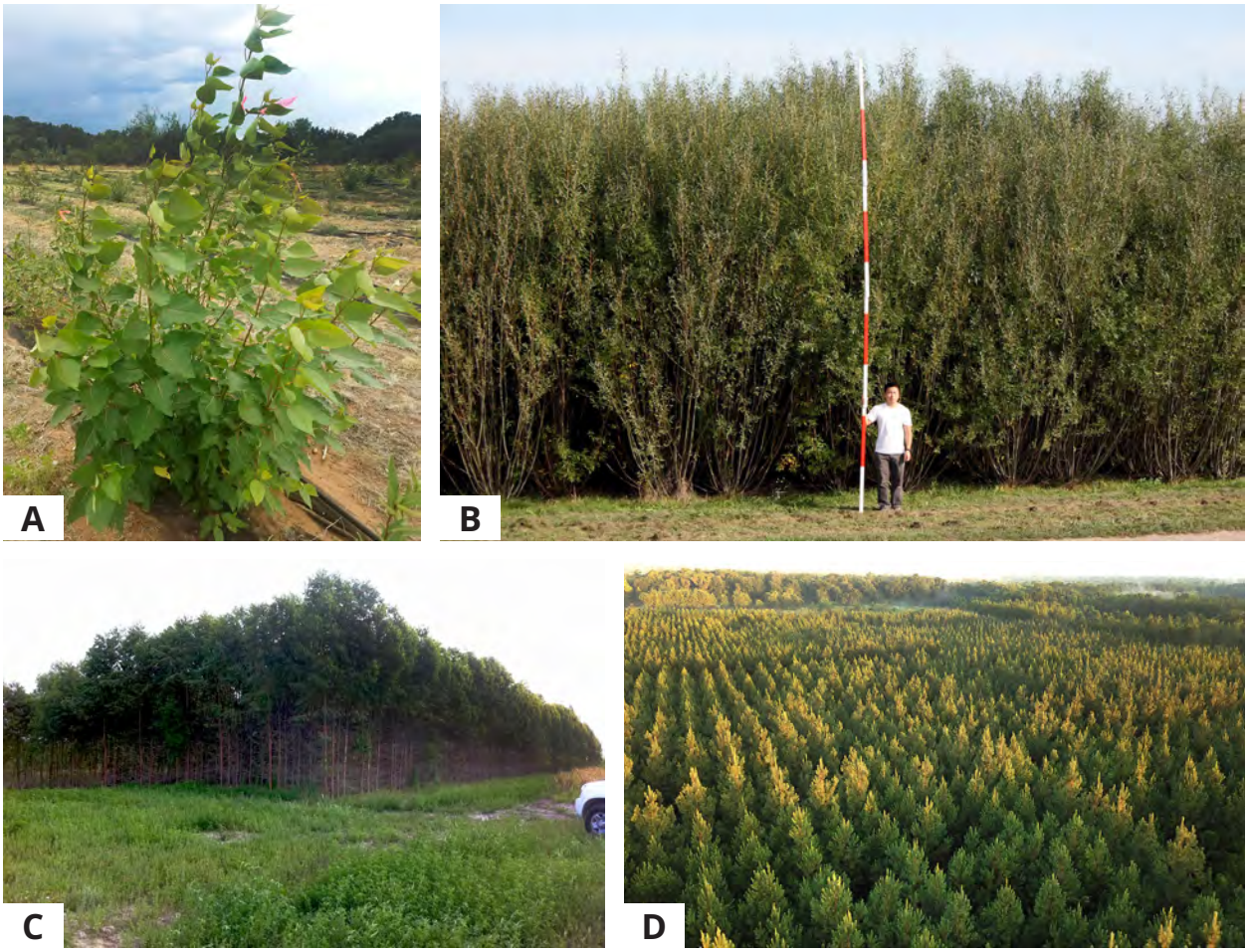

Fig. 2.4. High Biomass Productivity and Adaptability of Several Tree Genera Make Them Promising Candidate Bioenergy Feedstocks. (A) Drought-tolerant *Populus trichocarpa x Populus deltoides* hybrid early in its third growing season at a field site near Sedona, Ariz. **(B)** Mature three-year-old growth of new shrub willow cultivars in a selection trial growing at Cornell AgriTech in Geneva, N.Y. **(C)** Two-year-old stand of *Eucalyptus grandis* cultivars in central Florida. **(D)** Nine-year-old clonal loblolly pine in northern Florida. [(A) Courtesy Oak Ridge National Laboratory; (B) Reprinted with permission from Springer Nature from *BioEnergy Research* **8**(2) and Cornell University; (C) Courtesy Donald L. Rockwood, University of Florida and Florida FGT; (D) Courtesy Timothy A. Martin and Connor Bass, University of Florida]

Tree-based bioenergy systems must be capable of withstanding complex and often simultaneous stressors, all while sustaining productivity over many years.

growth, can be produced in diverse environments and production systems, and are flexible in terms of management (e.g., harvest times). These characteristics have generated interest in using them in large-scale dedicated bioenergy production systems growing specifically designed germplasm.

Poplars (*Populus* species and their interspecific hybrids) are well adapted for production in the U.S. Southeast, North, Pacific Northwest, and Midwest

in environments with consistent water availability. However, poplars are generally sensitive to drought stress (Rosso et al. 2023), with considerable variation in drought tolerance among species. For example, eastern cottonwood poplar (*Populus deltoides*) is much more drought tolerant than western black cottonwood (*Populus trichocarpa*): at full turgor, its osmotic potential is 0.4 megapascal lower than *P. trichocarpa* and its capacity for osmotic adjustment to drought stress is greater (Tschaplinski et al. 2006). Interspecific hybridization is now being used to combine traits for drought tolerance with the high productivity characteristics of certain genotypes.

Willows, like poplars, are easy to propagate and regrow rapidly after coppicing, which is the process of periodically harvesting aerial biomass by cutting tree shoots down to a low stump, then allowing the stump to regrow new shoots for future harvests. Willows perform well in cool regions with abundant precipitation, making them suitable for the U.S. Northeast and upper Midwest. Generally, species exhibit high cold tolerance and perform well on poorly drained soils and in floodplains. However, willows are susceptible to limited water availability (Savage and Cavender-Bares 2011).

Eucalyptus are woody plants that include several species native to Australia and Indonesia but are now planted in many regions of the globe. They grow rapidly in tropical and subtropical regions and can produce high biomass yields in short rotation cycles. Although the geographic range for successful production of *Eucalyptus* species and their hybrids is limited by slower growth at cooler temperatures and sensitivity to severe frosts (Oberschelp et al. 2020), identifying productive, cold-resistant species and hybrids can extend their growth range northward. Additionally, *Eucalyptus* species can be managed by annually harvesting coppice regrowth for biomass and bioproduct (e.g., terpene) production, which capitalizes on the root system being more resistant to cold than the shoot.

Among *Pinus* species, loblolly pine (*Pinus taeda*)—already critical to the lumber and paper industries—is a good candidate for woody bioenergy feedstock production. This species is native to the Southeast, with a production range largely limited by minimum winter temperatures (Matallana-Ramirez et al. 2021).

Several fast-growing tree species are well suited for short-rotation bioenergy feedstock production, with regional suitability largely determined by temperature and water availability.

Genomic Resources for Predictive Breeding of Stress-Resilient Trees

Important genetic and genomic resources supporting research and improvement of numerous tree species have been developed, including reference genome sequences (Tuskan et al. 2006; Nystedt et al. 2013; Zimin et al. 2014), diversity panels (Evans et al. 2014; Fahrenkrog et al. 2017), and breeding populations for genomics-assisted breeding (Resende et al. 2012). This resource investment has been especially notable for poplars. In 2006, the first tree species to have its entire genome sequenced—*P. trichocarpa*—provided a foundation for tree functional genomics.

Since then, the genomes of multiple *Populus* species have been characterized, and large *Populus* diversity panels encompassing natural variants from across the species range have been sequenced to identify alleles underlying adaptive traits to abiotic stressors. Recent studies mining the diversity panels have begun to pinpoint genetic loci controlling key ecophysiological traits. For example, genome-wide association studies and climate association analyses of a diversity panel of more than 1,300 *P. trichocarpa* genotypes grown under control and drought conditions identified loci that underlie variation in stomatal traits and climate of origin (Klein et al. 2025). This and other studies are establishing a foundation for marker-assisted selection of resource-efficient *Populus* varieties.

Future research should apply these findings to breeding, genetics, and mechanistic studies. The development of

scalable, predictive models that incorporate processes and hierarchies across scales and integrate environmental data is essential for guiding future tree research and deployment in commercial plantations. Advanced modeling platforms should leverage AI and ML to analyze transcriptomic and phenomic datasets, with the goal of identifying and developing productive genotypes with high resource-use efficiency and stress tolerance. Efforts to develop such integrative approaches—those that combine multiple layers of omics data (e.g., genomic, transcriptomic, proteomic, and metabolomic) with drought resilience properties—are currently underway for *Populus* (Weighill et al. 2019). In addition, advances in robotics and automated imaging are being used to precisely monitor poplar plants grown under controlled stress environments. Combining predictive modeling, omics data, and advanced automated phenotyping tools is expected to enable faster genetic gain—a measure of improvement in the genetic value of certain traits in a population—by enabling early identification of genotypes with superior performance under abiotic stress conditions.

Dissecting Stress-Response Networks To Engineer Tree Resilience

Stress tolerance and recovery are underpinned by complex molecular and physiological processes involving interactions among multiple genes, many of which are part of redundant or compensatory pathways. Disentangling these networks through transcriptomic, proteomic, and phenotypic analyses, complemented by quantitative genetic approaches, is essential to guide both classical breeding and molecular engineering strategies.

Dissecting molecular networks underpinning stress tolerance and recovery will lay the foundation for targeted bioengineering approaches. Biotechnologists can potentially eliminate tree vulnerabilities and enhance their durable resistance by identifying and targeting specific susceptibility genes for removal or modification using genome editing technologies. CRISPR-Cas9 and related genome editing systems are now routinely applied in poplar functional gene studies and to introduce desirable trait modifications. Notably, several

Dissecting molecular networks underpinning stress tolerance and recovery will lay the foundation for targeted bioengineering approaches.

proof-of-concept studies have shown that editing poplar genes can produce significant gains in traits relevant to bioenergy. Several pathogen susceptibility-associated loci have been identified in *Populus* (Muchero et al. 2018), defining candidate targets to improve plant resistance using genome editing. Researchers are also exploring harnessing symbiotic microbes that naturally enhance tree hardiness. Poplar trees host rich communities of endophytic bacteria and mycorrhizal fungi, some of which can confer tolerance to drought and other stressors. Thus, synthetic microbial communities that promote poplar growth and resilience are being developed, and genes that control the ability to form associations with beneficial soil microorganisms are being identified (Wu, J., et al. 2025).

Key research questions about molecular stress-response networks include:

- What regulatory elements, genes, and metabolites govern recovery from acute stressors such as temperature extremes?
- Can CRISPR-based activation or interference libraries be used in tree species to identify novel regulatory pathways controlling resilience traits?
- How does genetic redundancy affect trait expression across spatial scales (e.g., cell, tissue, and whole plant), and how can it be exploited in breeding programs?

Understanding Resource Acquisition and Allocation by Trees To Enhance Productivity and Stress Resilience

Scaling up from greenhouse experiments to field- and landscape-level trials is a high-priority need that

enables researchers to assess how stress-response traits translate across environments and predict resilience under future climate scenarios. Scaling to real-world conditions requires addressing questions across organizational and temporal scales, including how tree-based bioenergy production systems function long term.

Fundamental questions remain about the molecular and regulatory mechanisms that determine how trees acquire, transport, reallocate, and leverage nutrients and how these processes interact with root–microbe partnerships and affect stress resilience. While seasonal shifts in these mechanisms are known, variations across genotypes and environments are still being uncovered. Long-distance transport by xylem and phloem are known to respond to internal nitrogen status, hormonal cues, and source–sink dynamics, but how these mechanisms function across developmental stage or stress type is unknown. Furthermore, they are expected to vary widely among genotypes, creating a need to understand their genetic controls.

Similarly, the long-term effects of repeated harvest in a coppicing system remain uncertain. Unlike harvesting plant stems, tree coppicing preserves persistent belowground biomass, which serves as a reservoir for resources that are remobilized in the next growth cycle. Quantifying belowground dynamics, such as root turnover and mycorrhizal nutrient exchange, is challenging. However, empirical studies indicate that short-rotation poplar coppices can build up soil carbon stocks through root-derived inputs (Berhongaray et al. 2025). Also beginning to emerge are insights into how mycorrhizal fungi, endophytes, and rhizosphere bacteria influence nutrient acquisition and stress tolerance, the genetic determinants of host compatibility, and the mechanistic bases of microbe-mediated resilience.

Critical aspects and processes of tree productivity, stress resilience, and long-term persistence that require further study include:

- Seasonal changes in nutrient uptake and allocation.
- Mechanisms and control of long-distance nutrient transport.
- Nutrient mobilization, especially nitrogen, out of shoots at the end of the growing season.
- The effects of coppicing and harvest frequency on belowground responses, such as root growth and turnover, nutrient uptake, and regrowth dynamics.
- Root system interactions with endophytes, mycorrhizal fungi, and bacteria.
- Mechanisms underlying microbiome contributions to stress tolerance.

Scaling up growth, stress resilience, and long-term persistence to real-word conditions requires better understanding the processes governing resource acquisition and allocation in trees—within and across seasons and in response to stressors and coppicing practices.

2.5 Strategies and Opportunities To Improve Annual Crops

Meeting sustainable feedstock production goals will require cropping systems that integrate seamlessly into a variety of agricultural landscapes. Annual feedstock crops that can be deployed flexibly in rotations, either as primary summer crops or as intermediate offseason crops, offer promise for increasing energy, chemical, and bioproduct outputs and may even enhance food production (Gautam et al. 2026). In this context, sorghum and emerging oilseed crops represent complementary components of intensified, resilient feedstock systems, combining high biomass and/or seed oil yields with broad adaptability across diverse environments.

Sorghum as a Potential Bioenergy Crop

Annual grain crops, primarily corn, grain sorghum, and soybean, are important feedstocks in first-generation bioenergy production. Non-grain types of sorghum are now being considered in next-generation bioenergy feedstock production grown as a summer annual row crop (Mullet et al. 2014; Parikh et al. 2021; Park et al. 2024). Attractive attributes of this C_4 grass include

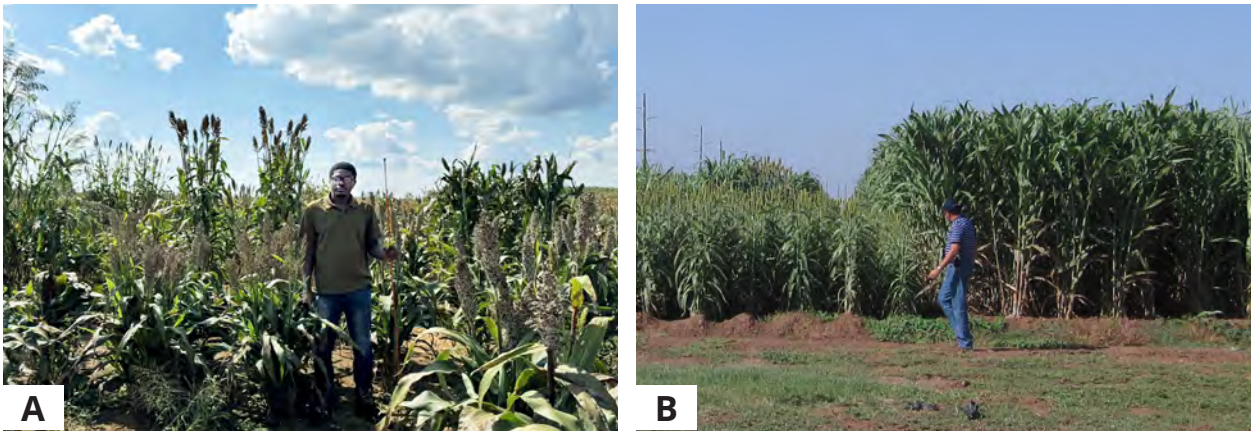

Fig. 2.5. Sorghum's Considerable Diversity and Genetic Resources Make it an Appealing Candidate for Bioenergy Crop Production. (A) The Sorghum Association Panel (Boatwright et al. 2022) planted at the Alabama A&M University Winfred Thomas Agricultural Research Station in Hazel Green, Ala., illustrates genetic variation in grain sorghum growth form, productivity, and grain production. **(B)** Various hybrids of photoperiod-sensitive sorghum, known as energy sorghum or biomass sorghum, in the Texas A&M AgriLife Research sorghum breeding program at the Texas A&M Agronomy Farm near College Station, Texas. In these sorghum types, flowering is delayed until late fall so they grow vegetatively until biomass harvest in early or mid-fall. [(A) Courtesy Alabama A&M University, (B) Courtesy Texas A&M University]

high biomass yields, natural drought and high temperature resilience, and extensive genotypic and phenotypic diversity.

Different types of sorghum, including grain and energy sorghums (e.g., sweet and high biomass), can be developed to satisfy distinct composition targets and end uses (Zheng et al. 2023; Nigam et al. 2025). As an annual crop, sorghum can readily be integrated into crop rotations and does not require an extended establishment period, providing producers with greater interannual flexibility than perennial crops. Sorghum can be grown in rotation with intermediate crops (e.g., oilseed) in cropping systems that could yield energy, chemicals, and bioproducts year-round (Gautam et al. 2025).

Sorghum's considerable genetic diversity (see Fig. 2.5, this page), coupled with genetic resources generated by the research community (e.g., high-quality reference genomes, large mutant populations, and a gene expression atlas), provide tremendous opportunities for efficient germplasm improvement and discovery of fundamental mechanisms that can be leveraged to enhance its stress tolerance (Shakoor et al. 2014; McCormick et al. 2017; Cooper et al. 2019; Tao et al. 2021; Boatwright et al. 2022; Morris et al. In Press). For sorghum to produce high yields of fermentable sugars on marginal lands, continued targeted efforts are needed to dissect key genetic and physiological processes to enhance biomass productivity, biomass composition, resource-use efficiency, and stress resilience (e.g., NUE, WUE, and carbon partitioning—including for root growth and exudation).

Rotating with Oilseeds as Intermediate Bioenergy Crops

More than 200 million acres of U.S. farmland is planted in annual row crops, mostly to produce food, animal feed, and fiber (NASS 2023). During the offseason, more than 90% of that farmland lies fallow because few intermediate crops fit in the time window between the primary crops' growing seasons to produce three cash crops in two years. Oilseed crops, such as pennycress (*Thlaspi arvense*), camelina (*Camelina sativa*), Ethiopian mustard (*Brassica carinata*), and oilseed rape (*Brassica napus*), exhibit considerable potential as intermediate crops that produce seed oil suitable

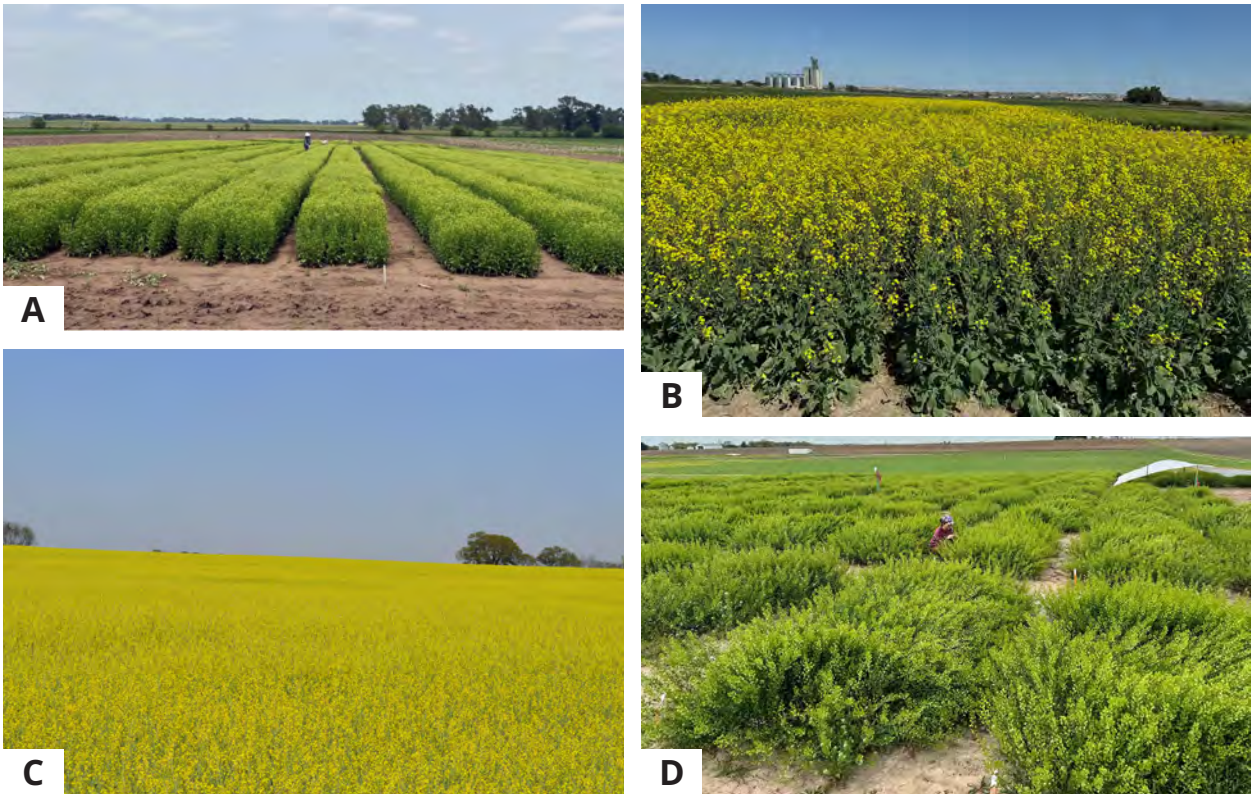

Fig. 2.6. Oilseed Annual Species Grown as Intermediate Bioenergy Crops May Enable Year-Round Bioenergy Production. **(A)** Camelina (*Camelina sativa*) grown at the University of Nebraska–Lincoln Biotech Field at the Eastern Nebraska Research, Extension, and Education Center in Mead, Neb. **(B)** Spring canola grown at the Eastern Agricultural Research Center, Montana State University, Sidney, Mont. **(C)** Flowering carinata (*Brassica carinata*) field at Tommy Dollar's Farm in Donalsonville, Ga. **(D)** Pennycress (*Thlaspi arvense*) grown near Macomb, Ill. [(A) Courtesy University of Nebraska–Lincoln; (B) Courtesy Chengci Chen; (C) Reprinted under a Creative Commons Attribution 4.0 International license (CC BY 4.0) from digital abstract of George, S., et al. 2021. "A Regional Interdisciplinary Partnership Focusing on the Development of a Carinata-Centered Bioeconomy," *Global Change Biology Bioenergy* **13**(7), 1018–29. DOI:10.1111/gcbb.12828; (D) Courtesy John Sedbrook, Illinois State University]

for conversion to bioenergy (see Fig. 2.6, this page; Resurreccion et al. 2021; Nóia et al. 2022; Phippen et al. 2022; Keadle et al. 2023). These winter or spring annuals can potentially be grown on millions of acres of fallow row crop farmland throughout the United States and produce billions of gallons of seed oil annually.

Sustainable intensification can increase production agriculture profits and contribute to the economic health of rural communities while providing ecosystem benefits without compromising food production. If oilseed crops were grown on 50 million acres of farmland (less than one-third of the total annual acreage in corn and soybean in the United States; NASS 2025) and produced approximately 60 gallons of seed oil per acre (1,500 pounds of grain per acre at 31% oil content; Sedbrook et al. 2014), annual production would equal 3 billion gallons of fuel plus feed and industrial coproducts to support the bioeconomy.

Work has only recently begun on characterizing intermediate oilseed crop species responses to abiotic stressors including drought, heat, cold, waterlogging, and nutrient deficiencies (Heydarian et al. 2018; Sanehkoori et al. 2021; Combs-Giroir et al. 2025; Srivastava et al. 2025). Moreover, as development of these crops proceeds, changes to the stress responsiveness of new varieties will undoubtedly occur and must be understood and

Key Takeaways for Improving Feedstock Crops

- Feedstock crop productivity is strongly influenced by resource availability and the interplay of environmental factors across crop life cycles.
- Abiotic stressors—including salinity, low soil fertility, and extremes in water availability, temperature, and pH—constrain productivity individually and in combination.
- Improving feedstock crop productivity requires a better understanding of the genetic, molecular, and physiological processes governing plant responses to abiotic stress.
- Research is needed to characterize stress responses across developmental stages and levels of biological organization, under individual and combined stressors.
- Advances in omics, phenotyping, and AI/ML-assisted modeling should be leveraged to better understand stress response mechanisms and guide design of improved genotypes.
- Developing and preserving diverse genetic and germplasm resources are central to achieving feedstock crop improvement through breeding, genome editing, and mechanistic studies.
- Field-scale experimentation and validation of controlled-environment research are essential to ensuring that discoveries translate to agricultural performance.
- Multilocation field trials across diverse environments are needed to study plant responses and their interactions with environmental factors and management practices.
- Multiseason experiments are essential to understanding seasonal resource allocation and the long-term effects of stress and repeated harvests on productivity and persistence of trees and perennial grasses.
- Experimental field trial locations should integrate advanced sensing modalities for high-resolution above- and belowground phenotyping and comprehensive environmental monitoring to support modeling and predictive breeding.
- Standardized data acquisition using replicable methods that are widely used within DOE is needed to ensure reproducibility and seamless integration into AI/ML pipelines.

targeted for improvement (Rasoul et al. 2025; Gautam et al. 2026; Jawahir et al. 2026). In addition to the oilseed species named above, other species (e.g., from the *Lepidium* genus) may also have untapped potential in U.S. bioenergy production (Ortiz et al. 2020).

Despite the new establishment of carinata, camelina, pennycress, and canola as viable intermediate oilseed crops and the known potential of sorghum as a bioenergy crop, uncertainty remains regarding geographic ranges for growth and optimal crop rotations for economical production. Limitations are based on a crop's ability to consistently germinate and establish; survive freezing, drought, and other stressors; and fit within a given growing season and crop rotation.

Leveraging Germplasm and Genetic Resources To Improve Vigor, Stress Resilience, and Productivity

Fully realizing the agronomic potential of these feedstock cropping systems requires exploiting the substantial natural and engineered genetic diversity present in sorghum and intermediate oilseed crops. Integrated phenotypic, genetic, and omics approaches are essential for identifying stress-resilient variants and

translating mechanistic insights into improved cultivars for diverse and marginal environments.

Considerable natural variation is present in the aforementioned annual crop species, including accessions adapted to different environments around the world (Mace et al. 2020; Li et al. 2021; Tao et al. 2021). Germplasm collections for these species are under various stages of phenotypic and omics characterization (e.g., Panelo et al. 2024). Researchers also have access to mutant collections (e.g., ethyl methanesulfonate mutant gene indexed populations that allow forward and reverse genetic analyses), targeted mutant lines (e.g., those created by CRISPR genome editing), and elite germplasm related to commercial varieties (Chopra et al. 2018; Demieville et al. 2025; Gautam et al. 2026). Detailed characterizations, primarily under controlled conditions, have yielded important insights related to microbial interactions and abiotic stress responses (Nishchenko and Hasanuzzaman 2020; Combs-Giroir et al. 2024; Zheng et al. 2024; Spishakoff et al. 2025). However, substantial opportunities remain in identifying natural variants and mutant combinations that shed light on fundamental mechanisms of stress responses. Such developments can pave the way for marker-assisted breeding and biodesign approaches that improve stress resilience.

The limited information available for several intermediate oilseed crops necessitates ongoing extensive phenotypic and omics characterizations of above- and belowground growth coupled with quantitative genetic approaches to identify advantageous genotypes (e.g., overall vigor and productivity as well as tolerance to diverse abiotic and biotic stressors). Characterization under controlled environmental conditions can enable rapid progress, especially in high-throughput facilities. However, given that many phenotypes observed under growth chamber conditions do not translate to the field, and some phenotypes do not manifest except under field conditions, diverse germplasm must also be evaluated in the field.

Acquisition of growth, physiological, and omics data from genetically diverse germplasm exposed to meaningful stress conditions provides important datasets for training AI/ML algorithms. This process also provides statistical power for deciphering fundamental stress response mechanisms and discovering stress- and yield-related natural and induced variants. New discoveries and hypotheses lead to the identification and generation of new genetics for interrogation.

Substantial genetic variation coupled with extensive phenotypic and omics characterization can be leveraged to elucidate fundamental mechanisms of stress responses and accelerate breeding and biodesign of more productive and resilient crops.

Industrial partnerships facilitate access to evolving elite germplasm to determine changes in stress responsiveness and vulnerabilities in commercial lines. For example, pennycress is undergoing rapid domestication, with major changes occurring in secondary metabolite pathways (e.g., reduced glucosinolates), metabolic fluxes to different seed components (higher oil and protein, less fiber, and reduced polyunsaturated fatty acids), and plant growth characteristics (e.g., changes to maturity, grain yields, and harvest index; Chopra et al. 2020; Rasoul et al. 2025; Gautam et al. 2026). Opportunities abound for deciphering how these rapid changes affect stress responses and microbial interactions. Care must be taken to assess and catalog beneficial traits to avoid losing them from commercial gene pools as breeding efforts progress.

Major research needs and opportunities to develop germplasm and genetic resources for annual feedstock crops include:

- Improving crop germination, establishment, and growth on marginal soils and under marginal conditions.
- Interrogating natural variation to identify fundamental stress response mechanisms and beneficial genetic variants.

- Understanding and mediating effects of stress on tissue composition.
- Enhancing abiotic stress resilience to improve yields.
- Improving water- and nutrient-use efficiencies to make better use of natural resources and expand production on marginal lands.
- Optimizing root architecture and function for improved water and nutrient uptake, soil organic matter formation, and microbial interactions.
- Improving photosynthetic efficiencies.
- Understanding interactions between abiotic stress conditions, particularly water and nutrient limitation.
- Understanding and tailoring phenology to increase stress resilience and adaptation to latitude and agronomic practices.
- Examining how genetic improvements in agronomic traits impact microbial interactions, abiotic stress responses, and resilience.

Chapter 3

Harnessing Plant–Microbe Interactions

Close interactions among plants and the microbial communities living in and around them—their microbiome—are increasingly recognized for their influence on crop yields and cropping system biogeochemistry. Plant microbiome composition changes rapidly (Ceja-Navarro et al. 2021) and can significantly benefit plant productivity, health, drought tolerance, and reproductive success (Mathur et al. 2006; Lau and Lennon 2012; Ortas et al. 2017; Lu et al. 2018; Trivedi et al. 2020). By some estimates, more than 25% of bacteria isolated from cultivated crops can promote growth in the host plant (Hassan et al. 2010; Marasco et al. 2012).

Microbes—including bacteria, fungi, protists, and viruses—form communities on or near roots (i.e., rhizosphere), inside leaves, stems, and roots (i.e., endosphere), and on leaf surfaces (i.e., phyllosphere; Bulgarelli et al. 2013; Xia et al. 2013). Classic examples of beneficial plant microbes include nitrogen (N)-fixing bacteria that enhance N availability and arbuscular mycorrhizal fungi (AMF) that increase water and nutrient uptake by plants (Averill et al. 2019). Plant microbiomes can confer resistance to disease, herbivory, and environmental stressors (Ghimire and Craven 2011; Giauque and Hawkes 2013; Straub et al. 2013a; Rho et al. 2018a; Giauque et al. 2019). Microbes can also regulate plant hormones and enhance seed germination and seedling establishment in annual crops (Rocha et al. 2019; Lamichhane et al. 2022). Plant microbiomes, including plant growth–promoting rhizobacteria (PGPR), can improve soil properties, such as water holding capacity and porosity, by influencing plant or microbial metabolite excretions

Beneficial plant–microbe interactions hold promise for supporting resilient crop production, but major gaps in understanding remain.

(Costa et al. 2018; Sher et al. 2020). Some endophytic microbes can defend the plant host from pathogenic microbes. The mechanisms underlying such protective effects are unclear but may include priming plant defense responses or direct inhibition through antimicrobials (Doty et al. 2023; Rabbee et al. 2024).

The complexity of community–environment interactions can complicate efforts to pinpoint the mechanisms that directly underlie beneficial plant–microbe interactions. New genomic technologies are introducing opportunities for novel mechanistic studies, but these have often been conducted in simplified model systems (Coleman-Derr and Tringe 2014).

Understanding the mechanisms by which microbes confer plant resilience and contribute to soil health is a major knowledge gap requiring additional research. While some microbial mechanisms may increase nutrient availability directly, such as N fixation and phosphate solubilization (see sidebar, Phosphate-Solubilizing Bacteria, p. 32), those that confer drought or thermal tolerance may be more indirect. In addition to outlining knowledge gaps, this chapter explores strategies to improve the beneficial characteristics of

Phosphate-Solubilizing Bacteria

Nitrogen fertilization confers the greatest impact on plant growth in short- to medium-term field studies (Muir et al. 2001; Kering et al. 2012, 2013), but phosphorus fertilization is required to maintain high yields over the long term (i.e., decades). Global high-quality mineral sources of phosphorus are rapidly being depleted (Cordell et al. 2009; Vaccari et al. 2009), and phosphorus runoff associated with fertilizer application is a major driver of environmental degradation via eutrophication.

While phosphorus is abundant in soils, only 0.1% is directly accessible to plants (Sharma et al. 2013). Many plant-associated bacteria can solubilize phosphate bound to soil minerals, increasing plant and fungal access (Rodríguez and Fraga 1999; Varga et al. 2020; Ding et al. 2021; Pan and Cai 2023). In turn, both arbuscular and ectomycorrhizal fungi can increase phosphorus accessibility to plants by extending the nutrient absorptive surface area of roots and producing organic acids, siderophores, and extracellular phosphatases (Bücking and Heyser 2003; Vance et al. 2003; Shen et al. 2011). Additionally, some bacteria can improve mycorrhizal colonization of plants (Artursson et al. 2005).

Harnessing phosphate-solubilizing bacteria in the plant microbiome could improve phosphorus recycling, retention efficiency, and bioavailability to plants (Sharma et al. 2013; Alori et al. 2017; Etesami et al. 2021). However, the precise role of the plant microbiome in determining the fate of phosphorus and the impact of the microbiome on soil phosphorus chemistry and availability to plants is poorly understood.

the plant microbiome and identifies opportunities for research in specific areas.

3.1 Key Knowledge Gaps in Plant–Microbe Interactions

Beneficial plant–microbe interactions hold promise for supporting resilient crop production, but major gaps in understanding remain, including mechanisms of communication, plant trait development, microbiome recruitment, environmental stressor impacts, and multifactor interactions in field settings (see Fig. 3.1, p. 33).

Metabolic Cross Talk

Plants and microbes communicate through metabolite exchange and manipulation (i.e., metabolic signaling), exerting influence over soil nitrous oxide fluxes and plant–microbe relationships under nutrient stress (Baker et al. 2024). The mechanisms of these relationships are well understood in well-studied plant–microbe symbioses (e.g., with rhizobia or AMF; Oldroyd 2013) and are triggered by known signaling molecules (e.g., lipochitooligosaccharides and strigolactones). However, metabolic cross talk between plant roots and the broader rhizosphere microbiome is less well characterized.

Relatively few studies have measured plant-root gene transcripts or exuded metabolites, and the corresponding responses of rhizosphere microorganisms, measured by gene expression. In sorghum, drought-linked shifts in root-associated soil microbial communities appear to be driven by changes in plant metabolites and microbial gene expression associated with (1) carbohydrate and amino acid transport and metabolism and (2) secondary metabolite biosynthesis (Xu, L., et al. 2018). In sorghum roots, changes in gene expression related to mineral homeostasis, hormone signaling, and osmotic regulation have been linked to rhizobiome changes during drought stress (Kabir et al. 2025).

In turn, microbial traits can alter plant gene expression. For example, foliar fungal endophytes changed plant

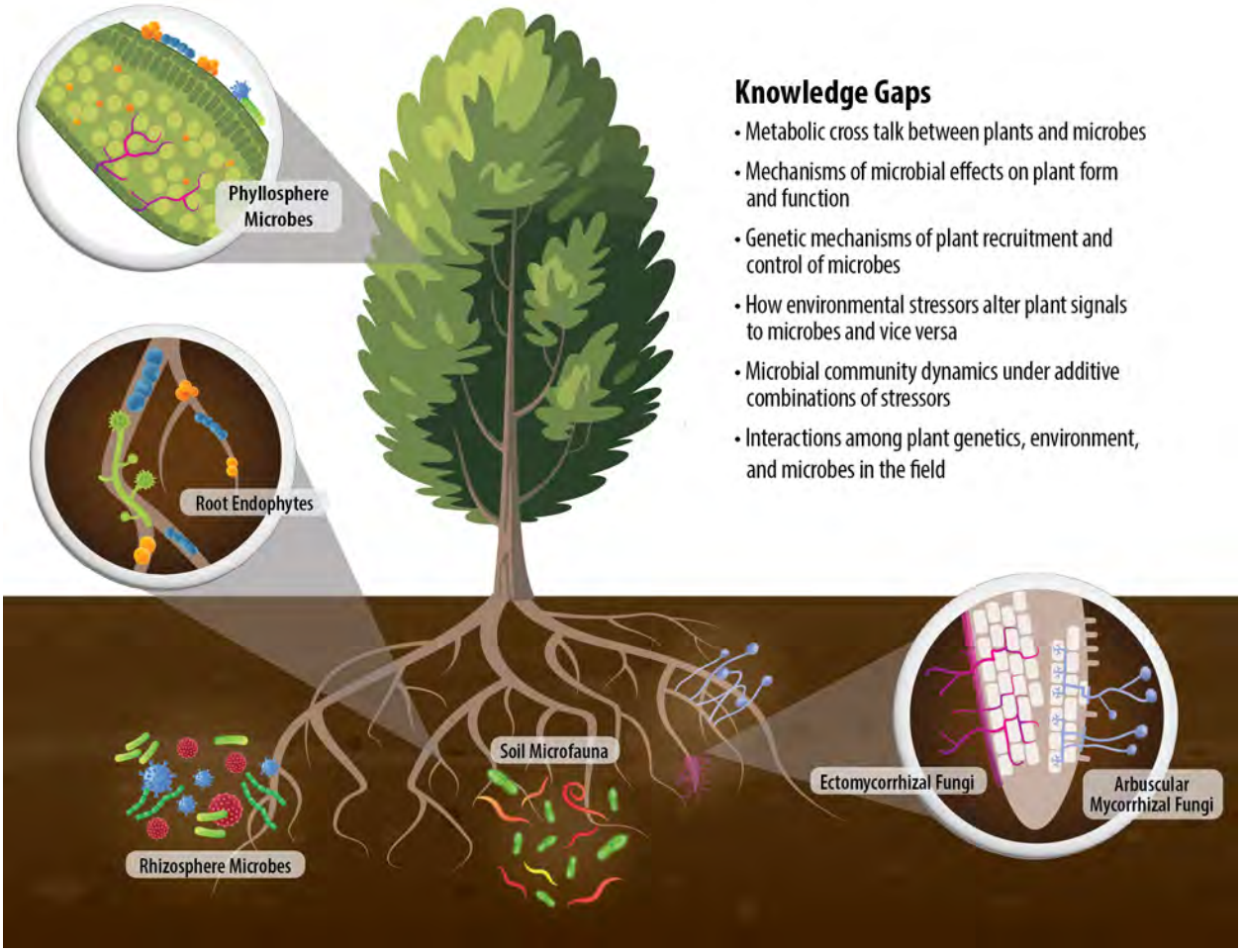

Fig. 3.1. Major Knowledge Gaps in the Plant–Microbe System. The plant–microbe system, known as the phytobiome, comprises a host plant plus the symbiotic and free-living microorganisms living within (i.e., endophytes), on, and around it. Plant–microbe interactions are concentrated in the phyllosphere, which includes all aboveground tissues of the plant (e.g., leaves, stems, flowers, and fruits), and in the rhizosphere, which includes the soil surrounding roots. Microbe–microbe interactions, as well as soil microfauna interactions with plants and microbes, can also play important roles in the phytobiome. Major knowledge gaps about plant–microbe interactions and how they contribute to plant resilience should be addressed if plant or microbiome engineering will be used to benefit resilient crop production.

gene expression in *Panicum hallii*, a genetic model for switchgrass, under water-stressed conditions (Aimone et al. 2023). However, much remains to be learned about how microbiomes can modulate host gene expression or phenotypic plasticity under stress conditions.

Microbial Effects on Plant Form and Function

Linking microbial mechanisms to specific impacts on plant form and function is another challenge. For example, evidence suggests the plant microbiome can enhance drought tolerance by promoting root proliferation, modulating stomata, and resetting the plant's osmotic potential (Rho et al. 2018b; Hartman and Tringe 2019; Porter and Sachs 2020), but the underlying mechanisms are poorly understood. The extent to which a plant's drought tolerance arises from host genetics, microbiome composition, or their interactions is an active area of research that incorporates microbes into traditional concepts such as the G x E x M equation, which examines how genetic potential (G), environmental conditions (E), and farm

management practices (M) interact to influence crop productivity (Oyserman et al. 2018, 2022).

Determining which plant traits are affected by microbes could inform microbial engineering approaches to support plant vigor and growth, as well as optimal timing and methods for microbial applications (e.g., seed coatings for better establishment or later applications for nutrient acquisition or biomass accumulation). New plant–microbe interaction studies are needed to determine whether persistent colonization is necessary or if targeted, stage-specific interventions are sufficient for crops to benefit from plant–microbe interactions.

Microbiome Recruitment and Biodesign

Plants have coevolved with diverse microorganisms—such as N-fixing bacteria and endophytic fungi—that support growth, nutrient acquisition, stress tolerance, and protection from pathogens (Redman et al. 2002; Wang, B., et al. 2016; Naseem et al. 2018; Carrión et al. 2019; Lanza et al. 2019; Compant et al. 2025). These microbial partnerships can expand plant functional traits to provide new avenues for agricultural management (Friesen et al. 2011; Hawkes et al. 2021).

How plants recruit and control their microbial partners and the genetic mechanisms that govern these interactions are major knowledge gaps. For example, legumes enrich both symbiotic and free-living N-fixing microbes, but understanding of the plant-produced signals that influence microbial recruitment is limited. Plants' ability to select beneficial microbes while avoiding pathogens is also poorly understood. Plant genotype may shape plant–microbe interactions (Berendsen et al. 2012; Edwards et al. 2023; Ji et al. 2023), but site or environment typically play larger roles. Studies need to better address genotype-to-phenotype links to determine when genotype matters (Peiffer et al. 2013; Whitaker et al. 2023). Moreover, microbiomes change rapidly as a plant grows (Shi et al. 2015; Nuccio et al. 2020), so repeated measurements are essential.

No single microorganism is uniquely important for plant health and resilience. For example, to cope with drought stress, plants recruit a diverse rhizosphere microbiome including AMF (see sidebar, Arbuscular Mycorrhizal Fungi, p. 35), beneficial rhizobacteria, and other soil microorganisms (Williams and de Vries 2020; Allsup et al. 2023; Kane et al. 2023). Interactions between AMF and free-living microbes appear responsible for enhancing plant performance by improving nutrient and water uptake and maintaining photosynthesis under drought conditions (Tang et al. 2022). Thus, plant microbiome engineering and management efforts should focus on community-level biodesign. More complex studies of the three-way interactions among plants, AMF, and other members of the soil microbiome are needed to understand key engagement by AMF with host plants and surrounding soil microbial consortia (Hestrin et al. 2022).

No single microorganism is uniquely important for plant health and resilience. Thus, plant microbiome engineering should focus on community-level biodesign.

Environmental Stressors

Abiotic stressors can alter rhizosphere composition and the root microbiome, causing both transient and lasting changes. Similar to drought, freeze–thaw cycles and extended winters alter microbiomes by changing water availability and the survivability of individual microbes (Acuña-Rodríguez et al. 2020). However, relatively little is known about the microbiome of feedstock grasses under stress, although two recent switchgrass studies report modest shifts in the rhizosphere microbiome under drought stress (Baker et al. 2024; Bandopadhyay et al. 2024).

A better understanding of how environmental stressors alter plant metabolites and exudates, which affect microbial recruitment and communication, is also needed. A disconnect exists between microbes identified as potentially beneficial and those actually recruited by plants, especially under stress.

Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal fungi (AMF) are abundant in the rhizosphere of most terrestrial plants, including the feedstock crops miscanthus (Barnes et al. 2016), switchgrass (Hestrin et al. 2021), and sorghum (Watts-Williams et al. 2021). They are highly influential due to their close associations with roots and their effects on plant performance and microbiome composition (see figure, Beneficial Arbuscular Mycorrhizal Fungi, next page; Delavaux et al. 2017; Changey et al. 2019). AMF act as nutrient and water conduits, exchanging plant carbon for inorganic nutrients and facilitating water transport (Wipf et al. 2019; Kakouridis et al. 2022).

AMF are particularly key to enhancing drought resilience in crops grown as biomass and seed oil feedstocks (Liu et al. 2015; Basyal and Emery 2021; Basyal and Walker 2023). For instance, inoculation with the AMF species *Rhizophagus irregularis* increased stomatal conductance in sorghum by nearly 50% under drought conditions (Symanczik et al. 2018), likely due to improved water foraging capacity (Kakouridis et al. 2022). Similarly, inoculating switchgrass with *Claroideoglomus etunicatum* resulted in a threefold increase in aboveground biomass under severe drought compared to uninoculated controls (Basyal and Walker 2023).

AMF also help channel plant-derived carbon to other soil microbes that produce enzymes for nutrient cycling (Kakouridis et al. 2024), thus increasing nutrient availability for plant uptake (Symanczik et al. 2018; Watts-Williams et al. 2021). This is particularly impactful in nutrient-poor soils where plants support more diverse AMF communities likely to aid in nutrient acquisition (Kane et al. 2023, 2024). Inoculating with AMF can enhance crop productivity under nutrient limitation (Sarkar et al. 2015) and under metal toxicity (Firmin et al. 2015).

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Interactions in Field Settings

A major gap exists in understanding interactions among plant genetics, the environment, and microbes in the field; studies in this area are resource intensive and complex. The persistence and resilience of beneficial microbes, especially under heat or drought stress, is not well characterized, nor is the plant host range preferred by microbes that confer stress tolerance.

The expression of beneficial microbial functions is highly dynamic and context dependent; most functional studies have been conducted under controlled rather than field conditions (Sieradzki et al. 2023a, 2023b). For example, numerous controlled studies have demonstrated that fungal and bacterial symbionts confer cold tolerance to plants by (1) improving antioxidant response, (2) increasing osmolyte concentrations, and (3) prompting early plant hormone signaling to induce rapid acclimation to low temperatures. However, evidence of these cold tolerance benefits in the field is mixed (Acuña-Rodríguez et al. 2020).

In field settings, microbial diversity and functionality may shift in response to previous crops, but little is known about how one crop affects the microbiome of subsequent crops in a crop rotation. In addition, although a few studies have examined the impacts of fertilizers on beneficial microbial communities, particularly on free-living N fixers in low-input perennial systems (e.g., Roley et al. 2018; Chen, H., et al. 2019; Bahulikar et al. 2020), further research is warranted.

Multiple Stressors

Stressor combinations, both simultaneous and sequential, are common under field conditions. However, microbial community dynamics under stress combinations, including additive combinations of stressors—such as drought, salinity, and high temperature—are

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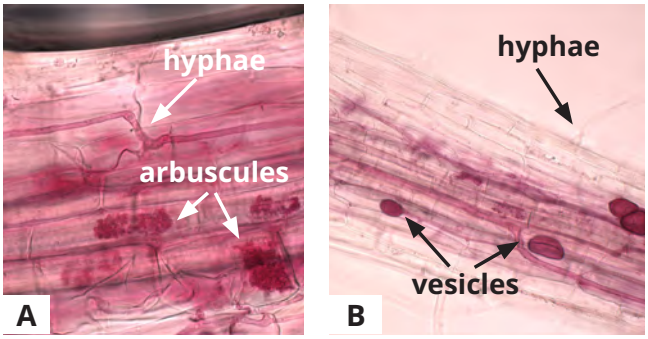
Metabolites released by AMF can recruit rhizobacteria, forming the foundation of multi-organism consortia in the rhizosphere (Kasanke et al. 2021). Recruiting beneficial rhizobacteria can enhance plant performance under stress (Bowles et al. 2018) by significantly improving stomatal conductance under drought stress, for example (Augé et al. 2015). AMF can promote plant growth and immunity and produce synergistic effects when paired with other beneficial microbes (Vannini et al. 2021). However, AMF species differ widely in their interactions with both plants and other soil microbes, ranging from mutualists that enhance plant performance to commensal or even parasitic species that provide little benefit (Kaur et al. 2022). Despite advances in understanding AMF, they are just one component of the complex root microbiome. Further research is needed to fully understand and harness beneficial functions of AMF in field settings.



Beneficial Arbuscular Mycorrhizal Fungi. Light microscope images show *Avena barbata* (wild oat) roots dyed with acid fuchsin showing *Rhizophagus intraradices* arbuscular mycorrhizal fungi (AMF) structures living inside the roots. **(A)** AMF arbuscules appear as highly branched fungal structures resembling trees, **(B)** AMF vesicles appear as dark red spheres, and hyphae appear as long, branched lines running the length of the lighter-stained roots. [(A) Reprinted under a Creative Commons Attribution-NonCommercial 4.0 International license (CC BY-NC 4.0) from Kakouridis, A., et al. 2024. "Arbuscular Mycorrhiza Convey Significant Plant Carbon to a Diverse Hyphosphere Microbial Food Web and Mineral-Associated Organic Matter," *New Phytologist* **242**(4), 1661–75. DOI:10.1111/nph.19560; (B) Courtesy University of California–Berkeley]

not well characterized. To advance crop resilience to stressor combinations, research needs to move from descriptive studies of microbial communities to a mechanistic understanding of microbial impacts. Microbial communities may play particularly important roles in soils experiencing frequent droughts and/or saltwater intrusions. For example, fungal interactions may be especially important for plant–water relations under drought and salt conditions (Worchel et al. 2013; Dastogeer 2018; Lanza et al. 2019; Aimone et al. 2023; Zaman et al. 2024).

3.2 Strategies To Improve Plant Microbiome Benefits

Strategies to harness the microbiome for resilient crop production may require altering plant microbiome composition and function to improve crop productivity and resilience (Coleman-Derr and Tringe 2014). One strategy involves plants augmenting their existing microbiomes by exerting evolutionary pressure on the microbial community (Mueller and Sachs 2015); altering feedbacks among plants, soil, and microbes; and manipulating the many drivers of community assembly (e.g., migration, selection, drift, and diversification). A second strategy introduces beneficial microbes—either individual strains or mixed consortia—to bioenergy cropping systems. These microbial inoculants (i.e., biologics) can be genetically engineered (e.g., gene editing N-fixing bacteria to activate biological N fixation in N-rich environments; Martinez-Feria et al. 2024) or isolated from natural populations conditioned to extreme environments. A third strategy improves soil health to recruit phylogenetically and

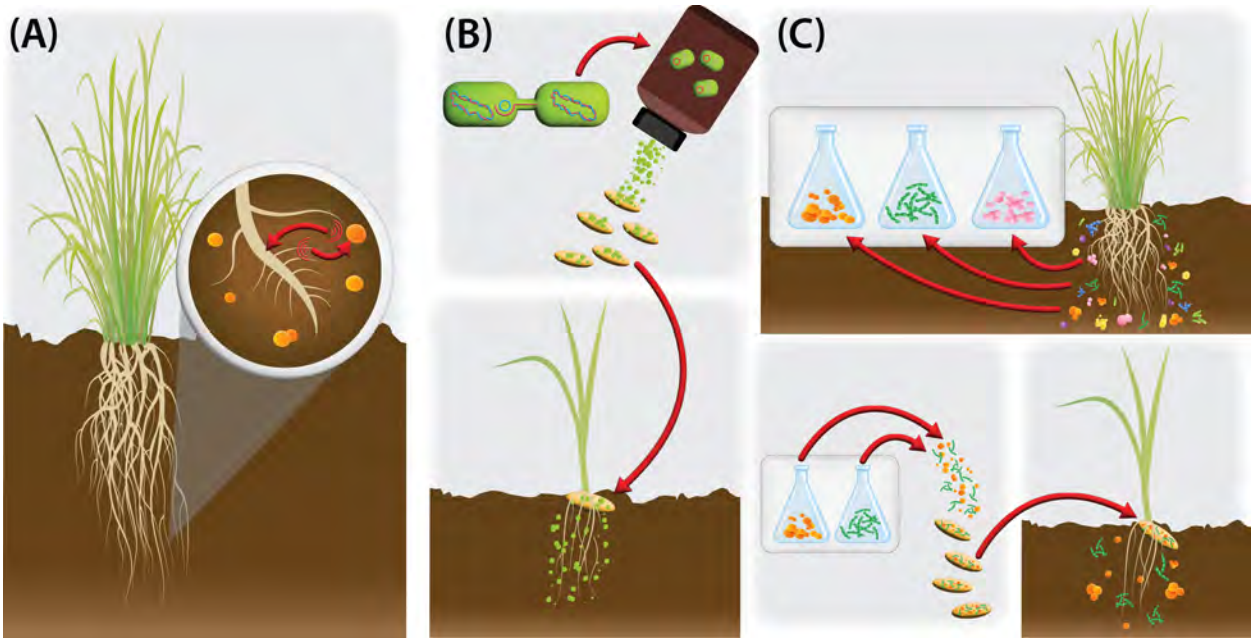


Fig. 3.2. Beneficial Plant Microbiomes Can Be Managed in Various Ways. (A) Plants can be bred or genetically engineered to recruit beneficial microbes. **(B)** Crops can be inoculated with microbes engineered to have beneficial functions. **(C)** Crops can be inoculated with synthetically assembled communities (SynComs) of beneficial microbes isolated from nature.

functionally diverse microbiomes, with benefits for crop productivity and resilience. Each strategy has different knowledge gaps that must be addressed to reach full potential.

Engineer Plants To Recruit Beneficial Microbes In Situ

Research indicates that plant genotypes can actively shape their microbiomes by secreting specific root exudates (Zhalnina et al. 2018; Williams and de Vries 2020; McLaughlin et al. 2023), suggesting the possibility of breeding or engineering crops to preferentially associate with beneficial microbial consortia (see Fig. 3.2A, this page). Understanding how plant traits select for beneficial microbes and identifying plant gene variants that enhance beneficial plant–microbe interactions are vital to realizing that possibility. However, significant knowledge gaps exist about the identity of plant root exudates and other signaling mechanisms that could be manipulated to improve plant–microbe interactions and the persistence of beneficial microbes.

The immense diversity of molecular signals produced by plants complicates efforts to target and tune specific microbial responses.

A major obstacle to leveraging plant–microbe signaling is the immense diversity of molecular signals produced by plants, which complicates efforts to target and tune specific microbial processes and requires advances in instrumentation and methods for analyzing metabolites (see Section 5.5: Instrumentation Opportunities and Needs, p. 59). Simplified synthetic communities and model plant systems are valuable for studying signaling and interactions, but translating these findings to complex field systems remains difficult.

In addition to plant chemistry, root architecture may influence microbial colonization. To guide plant breeding and selection of beneficial microbiomes, studies are needed to correlate effective colonization with root architecture among different feedstock crop species. How engineered root traits affect microbiome assembly and function is also unknown, and the feedback dynamics between plant development, root architecture, and microbial colonization must be better understood.

Although the potential exists to engineer beneficial microbial communities via plant manipulation to enhance crop resilience, this field is still in its early stages. Plant traits selected for enhanced resilience must be precisely targeted to reduce the possibility that promoting one trait inadvertently inhibits others. A deeper understanding of the pathways plants use to control their microbiomes could lead to more effective strategies for harnessing beneficial plant–microbe interactions *in situ*, thereby adding plant-mediated recruitment approaches to the direct microbial inoculation approaches discussed in the next section.

Amend Crops with Engineered Microbes

Beneficial microbes can be introduced to cropping systems to promote growth, disease resistance, and resilience against a range of environmental challenges. However, microbial amendment strategies should consider plant genetic resistance to pathogens, including the potential need to monitor plants' defense response against pathogens and nonbeneficial microorganisms that may be introduced.

Gene editing that is based on a fundamental understanding of the mechanisms by which microbes confer crop benefits could generate beneficial microbial inoculants (see Fig. 3.2B, p. 37). Another report in the Frontier Science for the Bioeconomy series, *Engineering Microbial Communities*, focuses on the potential for engineered microbial communities to improve plant health and discusses how to assemble and characterize synthetic microbial communities in more detail (U.S. DOE 2026).

While it is possible to produce plant–microbe combinations through genetic engineering, regulatory and practical considerations may favor screening natural populations for beneficial traits. For example, harnessing conditioned microbiomes from extreme environments (e.g., desert or alpine ecosystems) could potentially improve resilience in novel production regions (see Fig. 3.2C, p. 37). However, understanding microbial adaptation to local environments is critical, as introduced microbes may not thrive outside their native context. In addition, more research is needed on interactions between diverse germplasm and microbial inoculants to evaluate the potential for a universal inoculum versus crop- or environment-specific inocula (Petipas et al. 2021; Khasanova et al. 2023). AMF are particularly promising as a microbial inoculant because they are preadapted and evolutionarily predisposed to be beneficial symbionts. However, the successful colonization of roots by AMF inoculants and subsequent establishment of a symbiotic relationship remains a major challenge (Koziol et al. 2024; Boussageon et al. 2025).

New approaches are needed to achieve establishment, long-term persistence, and efficacy of microbial inoculants for nonfood feedstock crops.

Insufficient knowledge of successful ways to achieve establishment, long-term persistence, and efficacy of microbial inoculants is a major challenge to their use with crops grown as biomass or seed oil feedstocks (Koziol et al. 2024). For example, introducing microbial inoculants into already diverse and competitive soil environments is particularly difficult because new microbes may not establish and proliferate. This is especially true for endophytes (see sidebar, Endophytes, p. 39), for which the most effective timing, frequency, and concentration of inoculation necessary to achieve desired plant-level effects has not been determined.

Timing, application method, and type of cropping system are critical considerations in the establishment and persistence of inoculated microbes in a crop environment. Application methods are diverse and require further development, from effective ways to inoculate

Endophytes

Endophytes—microbes residing within plant tissues—include both bacteria and fungi, each with distinct and shared benefits to the plant host. Endophytes may be an especially important part of the feedstock crop microbiome, as their ability to persist (e.g., in roots and seeds) could underlie ongoing stress adaptation (Rodriguez et al. 2009); however, they are understudied.

Bacterial and fungal endophytes associated with plant roots can (1) enhance resilience against nutrient limitation by increasing nutrient supply to plants and (2) confer drought tolerance by altering host plant physiology (e.g., osmotic adjustment and root hydraulic conductivity). The mechanisms by which endophytes improve crop resilience are key knowledge gaps.

Unlike arbuscular mycorrhizal fungi (AMF), which are obligate symbionts, root endophytic fungi often are facultative partners that can also function as soil saprophytes—organisms that live on decaying organic matter. They produce a wide range of enzymes capable of breaking down soil organic matter (Knapp et al. 2018) and possess desiccation-resistant hyphae (Bell and Wheeler 1986). This suggests they may be less dependent on the plant host and more tolerant of drought than AMF. As a result, plant–fungal interactions are likely influenced by both soil organic matter levels and moisture stress, which affect decomposition and the transfer of plant carbon to the soil (Wang et al. 2021). Understanding how these relationships change under varying conditions will be crucial for adapting to abiotic stressors and predicting and enhancing microbe-mediated crop productivity on marginal soils.

Facilitating Nitrogen Supply

Some endophytic bacteria fix atmospheric nitrogen to its more bioavailable form, ammonium (Santi et al. 2013). Such diazotrophic bacteria are found in feedstock crops (see figure, Endophytic Microbes Benefit Plants, p. 40), including poplar (Knoth et al. 2014; Doty et al. 2016; Sher et al. 2024), sorghum (Sapkota et al. 2023), miscanthus (Kirchhof 2001; Davis et al. 2010), and switchgrass (Xu, J., et al. 2018). Through nitrogen fixation, diazotrophic endophytes can directly provide this essential nutrient. However, the mechanisms for optimum endophytic nitrogen fixation have not yet been determined.

Nitrogen can also be supplied through improved access via fungi associated with plant roots. Roots are inhabited by abundant endophytic fungi, which frequently co-occur with AMF (Lee and Hawkes 2021) and can even dominate environments with high soil organic matter (Kauppinen et al. 2014).

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seeds and explants in the laboratory to how to apply inoculum after planting in the field (e.g., via encapsulation technologies or foliar sprays).

Potential solutions to the challenges of achieving microbial persistence could include establishing cover crops to serve as microbial reservoirs that maintain populations for long-term soil health and plant resilience without the need to reinoculate. Microbial inoculants could also be applied exclusively during periods when beneficial plant–microbe interactions are needed. Automated monitoring systems could detect early indicators of stress to inform the timing of microbial interventions. More research is needed on scalable delivery methods for microbial inoculants at different stages of plant growth.

Scaling up successful laboratory or greenhouse inoculation strategies to large, variable field environments is another major obstacle because performance can be

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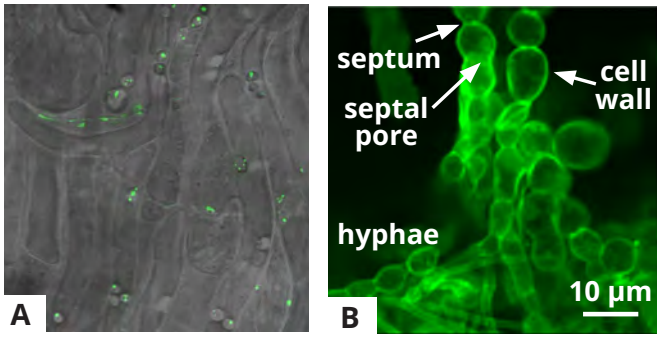
Altering Plant Physiology

Many endophytic microbes, as well as rhizosphere microbes, produce plant hormones or hormone-like metabolites that shape root development, plant growth, and stress responses (Eichmann et al. 2021). Auxin is the most widespread plant hormone and is produced by both bacteria (e.g., *Azospirillum, Azotobacter, Pseudomonas, Bacillus,* and *Rhizobium*) and fungi (e.g., *Trichoderma* and *Aspergillus)*. Cytokinins, gibberellins, and salicylic and jasmonic acids are similarly produced by a range of plant-associated bacteria (e.g., *Rhizobium, Methylobacterium, Pseudomonas, Azospirillum, Paenibacillus,* and *Bacillus*) and fungi (e.g., *Serendipita* and *Trichoderma*). Despite this diversity of hormone- and metabolite-producing microbes, it remains unclear which taxa meaningfully influence plant physiology or how microbial and plant hormone pathways interact. Similarly, the links between microbial metabolite production and root traits, community composition, and plant performance are not well established. The absence of mechanistic and predictive models is a major barrier to forecasting and engineering hormone-driven plant–microbe interactions.



Endophytic Microbes Benefit Plants. (A) Confocal microscopic imaging shows endophytic *Burkholderia vietnamiensis* (strain WPB) bacterial cells expressing the *nifH* gene inside *Populus trichocarpa* root epidermal cells to fix nitrogen for the plant. Expression of *nifH* (shown here in green fluorescence) is sometimes localized to spherical structures (i.e., nitrosomes), roughly 3 to 5 micrometers in size, inside epidermal cells in the root elongation zone. **(B)** A two-photon excitation fluorescence microscope image (µm, micrometers) shows the fungal hyphae of *Serendipita bescii*, a newly isolated symbiotic fungal species that improves plant growth even in drought conditions. [(A) Reprinted under a Creative Commons Attribution 4.0 International license (CC BY 4.0) from Sher, A. W., et al. 2024. "Dynamic Nitrogen Fixation in an Aerobic Endophyte of *Populus*," *The ISME Journal* **18**(1). DOI:10.1093/ismejo/wrad012; (B) Reprinted under a Creative Commons Attribution-NonCommercial-No Derivatives 4.0 International license (CC BY-NC-ND 4.0) from Lee, J., et al. 2022. "Label-Free Multiphoton Imaging of Microbes in Root, Mineral, and Soil Matrices with Time-Gated Coherent Raman and Fluorescence Lifetime Imaging," *Environmental Science & Technology* **56**(3), 1994-2008. DOI:10.1021/acs.est.1c05818]

inconsistent across different soils and climates (Russ et al. 2023). Furthermore, the safety of releasing engineered microbes into field settings has not been thoroughly studied, highlighting the need for controlled ecosystem trials before broader deployment.

Improve Soil Health

Although the concept of soil health is diffuse, with varied definitions and metrics (Fierer et al. 2021), seeking improvements in soil properties and processes that benefit crop yield and resilience is a generally accepted goal in feedstock crop production. Key metrics for soil health include soil organic matter content; carbon and N stocks; microbial biomass and diversity; fungal-to-bacterial ratio; microbial enzyme activities; and abundances of mycorrhizae, nitrifiers (see sidebar, Nitrifiers, p. 41), pathogens, and PGPR. More diverse belowground communities typically have

higher nutrient cycling rates and capacity to defend against pathogens. Crop management practices such as cover cropping, reduced tillage, and organic soil amendments have been widely observed to encourage microbial functional and phylogenetic diversity. Still, more rigorous and quantifiable measures of soil health should be developed.

Healthy soils benefit resilient crop production directly—by providing mineral nutrients and structure for plant growth—and indirectly—by encouraging the growth and recruitment of diverse beneficial microbial communities that provide nutrients and stress resilience to plants. Substantial evidence suggests that crop resilience to both abiotic and biotic stressors improves with increasing soil microbial diversity (Compant et al. 2025). However, a need exists to better understand (1) the mechanisms underlying this effect and (2) ways to encourage greater microbial phylogenetic and functional diversity in soil (e.g., through crop choice, management practices, and soil amendments).

In addition to improving soil microbial diversity, the plant microbiome can improve soil health by influencing soil structure and carbon fluxes. Mycorrhizal and root endophytic fungi, for example, can enhance soil structure by promoting soil aggregation (Hosseini et al. 2017). Enhanced soil structure benefits plant productivity and resilience by promoting root growth, improving water retention and gas exchange, and increasing soil microbial activity (Or et al. 2021).

In the context of soil carbon fluxes, root-associated fungal hyphae in the plant microbiome provide N, phosphorus, and water to their plant hosts and serve as pathways for transferring carbon from plant roots into the soil (Kaiser et al. 2015; See et al. 2022), thereby influencing soil organic carbon stocks—an important element of soil ecosystem processes and soil water holding capacity (Kopittke et al. 2022). A significant amount of plant-derived carbon is taken up by the microbial community associated with fungal hyphae (i.e., hyphosphere), which is compositionally distinct from the microbial community associated with the rhizosphere (Cheng et al. 2012; Nuccio et al. 2013; Kaiser et al. 2015). The hyphosphere may also stimulate the decomposition of soil organic matter, potentially through the release of hyphal exudates (Hooker et al. 2007; Toljander et al. 2007), providing nutrients to the host plant.

Nitrifiers

Nitrifiers are specialized microorganisms that drive transformation of ammonia to nitrate in the nitrogen cycle, profoundly negatively influencing soil fertility and plant nutrition. This functional group—comprising ammonia-oxidizing bacteria and archaea, nitrite-oxidizing bacteria, and complete ammonia oxidizers—can be remarkably abundant in agricultural soils (Leininger et al. 2006; Zhalnina et al. 2013).

Nitrifier activity governs the balance between nitrogen retention and nitrogen loss, shaping key processes such as nitrate leaching and nitrous oxide production. This group is responsible for 50% to 70% fertilizer loss in fields (Coskun et al. 2017).

Despite their importance, it is unclear how nitrifier populations assemble, compete, and persist in the highly dynamic and heterogeneous soil environment. Their interactions with plants are similarly obscure (Norton and Ouyang 2019). Some studies have shown that plants can exert control over nitrifiers by producing metabolites inhibiting their activity around plant roots. However, the mechanisms underlying these interactions and their consequences for nitrogen availability and nitrogen loss from agricultural systems are not well described.

Innovations in artificial intelligence and machine learning may identify trends in metabolic cross talk between plants and microbes.

3.3 Opportunities To Fill Knowledge Gaps and Support Microbiome Improvements

Thoughtful management of beneficial plant–microbe interactions may enable reliable biomass production with low inputs of fertilizer and water while maintaining key ecosystem processes, such as nutrient retention and soil organic matter formation. However, the research community is only beginning to understand (1) the often context-dependent mechanisms underlying plant–microbe interactions and microbiome assembly and (2) how to reliably harness crop plants' beneficial microbial partners (Trivedi et al. 2020).

While substantial research has been conducted on plant growth–promoting microorganisms, trials of their field-scale efficacy are limited (Compant et al. 2010), and industrial-scale bioamendment applications are relatively rare (Parnell et al. 2016). Efforts to fill these research gaps must be interdisciplinary and collaborative, involving plant biologists, microbiologists, and soil scientists. Both reductionist studies (i.e., using laboratory cultures and consortia) and studies of natural wild communities are needed. Research is also needed to prevent unintended consequences of microbial inoculants at the plant and ecosystem levels and to test delivery mechanisms, such as seed coatings (Rocha et al. 2019) and inoculation with leaf symbionts.

Various research opportunities, identified in this section, provide strategies to harness the microbiome by closing key knowledge gaps. These efforts enable selection of microbial taxa that benefit crop growth, physiology, nutrient uptake, and abiotic stress tolerance and ultimately improve feedstock crop resilience (Brejda et al. 1998; Ghimire et al. 2009).

Decode Plant–Microbe Cross Talk

Understanding which plant traits (e.g., root distribution, composition, and exudates) interact with microbes to mediate microbiome assembly, how these interactive plant traits vary, and their temporal stability is essential to manipulating the microbiome (Zhao et al. 2025). A significant need exists to better identify and annotate plant exudate diversity (e.g., auxin, ethylene, and volatile organic carbons) and the signaling mechanisms that select and maintain the plant microbiome (Baker et al. 2024). These plant signals may elicit microbiome feedbacks that influence crop growth (Straub et al. 2013a; Farrar et al. 2014), particularly under biotic or abiotic stress.

Deciphering these critical conversations will require mechanistic studies focused on biosynthetic processes and chemical signaling between plants and microbes, with an emphasis on quantitative omics and chemistry-focused approaches. Whether these processes are intentional plant strategies or products of evolutionary selection is an open question. Progress in this area may be accelerated by combining simplified experimental systems with advanced data analysis, including artificial intelligence and machine learning, to make sense of large complex datasets.

Study the Phytobiome

An important approach for future feedstock crop–microbiome research is to incorporate the microbiome into holistic plant adaptation strategies. This practice of viewing the plant and the symbiotic and free-living microbes living on, within, and around it (i.e., phytobiome) recognizes that the complete system may express synergistic traits that the plant and microbiome alone cannot (Schweitzer et al. 2008; Peiffer et al. 2013; Sánchez-Cañizares et al. 2017; Deng et al. 2021). Some studies have found that links between microbiome taxa and plant host genetics are heritable, suggesting it may be possible to select plant genotypes based on both plant traits and desirable microbial benefits (Peiffer et al. 2013). Other research suggests that nonfood feedstock crops, having experienced less breeding pressure than traditional crops, may retain essential microbiome

interactions that may be lost in more intensively bred cultivars (Farrar et al. 2014).

While most studies on plant–microbe interactions have focused on bacteria and fungi, it is important to consider the broader phytobiome, which includes protists, viruses, and soil microfauna. Some nematodes preferentially feed on N-fixing bacteria, influencing nutrient cycling. Additionally, while the roles of AMF and rhizobacteria are individually well understood (Duan et al. 2024), the complex interactions within AMF consortia and their collective impact on crop performance are less clear. As such, whole-consortia studies are needed to add realistic complexity to typical inoculation approaches that follow the "one microbe, one plant" model. New tree-of-life sequencing approaches are making whole-consortia studies tractable (Nuccio et al. 2021).

Studying the plant and all microbes living within, on, and around it as a "phytobiome" system will expand the scope of plant research by considering multiple interactions simultaneously.

Focus on Symbionts

Known plant symbionts (e.g., N-fixing rhizobia and AMF) are a logical first choice for feedstock crop bioaugmentation because their diverse genetic traits have coevolved with those of their plant hosts. Bioaugmentation with symbionts may be particularly ideal in arid, stressed conditions where they could confer multiple types of abiotic and biotic stress tolerance on host plants (Coleman-Derr and Tringe 2014).

Endosymbionts—organisms that live at least partially inside another organism and form a mutually beneficial relationship—are another logical focus because a considerable fraction of endophytes isolated from crops influence host plant fitness (Friesen et al. 2011). Microbe–microbe interactions must be studied both *in vitro* and *in planta* to understand the community-level mechanisms of benefits provided to host plants.

Identify Active Microbes and Their Functions

Genome reconstructions from shotgun sequencing data can reveal key metabolic capabilities and predict the roles of uncultured organisms in ecosystem processes (Straub et al. 2013b). However, metagenomics only describes the functional or metabolic potential of soil microbial communities. Identifying the active microbes in a system is prerequisite to revealing their functional roles. For example, certain microbes can help maintain plant productivity under drought conditions; others can suppress plant pathogens. The mechanisms behind these different functions are unclear. Advanced methods like quantitative stable isotope probing, metatranscriptomics, metaproteomics, and predictive tools for microbial function can help connect microbial sequence data to function. However, current databases for microbial function are incomplete, and the same sequence may not correspond to the same function across ecosystems.

Conduct Temporally Resolved Long-Term Field Studies

Controlled studies in simplified systems are needed to generate fundamental knowledge about plant–microbe interactions. However, these studies cannot capture the full spectrum of taxa, interactions, or variables in field settings. New ways to link small-scale experiments to large-scale processes must be developed in order to transfer knowledge gained in the laboratory or greenhouse to cropping systems in the field, where environmental conditions are temporally dynamic and crop rotations and land management influence outcomes.

Comparative microbiome studies across species and genotypes can help identify universal versus host-specific microbial interactions. Field-scale trials across multiple climatic zones can determine context-dependent outcomes. Common gardens have been used to determine the influence of plant genetics versus environment on plant phenotype, but the microbiome has rarely been considered in this context (Schweitzer et al. 2008; Gehring et al. 2017).

Long-term quantitative studies are needed across multiple seasons, crop rotations, and regions to track the

Key Research Opportunities To Harness the Microbiome in Resilient Crop Production

- Decode plant–microbe cross talk to understand how to manipulate signaling pathways (1) in microbes to alter plant form and function and (2) in plants to recruit beneficial microbes.
- Study the phytobiome (i.e., the plant and all microorganisms in its environment) as a single system that may express synergistic traits.
- Focus on bioaugmenting symbionts that have coevolved with plant hosts.
- Move beyond characterizing the genetic potential of the plant microbiome to identify active microbes and their beneficial functions.
- Conduct temporally resolved long-term field studies to generate fundamental understanding of plant–microbe interactions under temporally dynamic environmental conditions.
- Model plant–microbe interactions to identify plant and microbial biodesign targets for improved crop resilience.

effectiveness and persistence of microbial inocula. For example, it is unknown whether common microbes present during relay cropping determine how each crop affects the associated microbiome for the following crop. Some microbes may have a higher affinity for particular crop species and therefore exhibit higher stability over time in those cropping systems.

Understanding how to manipulate the right plant–microbe interactions at the right time is also important. For example, some microbes may benefit perennial crop establishment under marginal conditions and do not need to persist in the system beyond the crop establishment period, while other microbes are required for long-term resilience. Long-term microbiome analysis is needed to determine the impacts of abiotic stressors on persistence, informing the potential need for reinoculation with core microbiota. Current measurement schemes are not well suited to capturing both short- and long-term microbiome dynamics, making this knowledge gap difficult to address.

Model Plant–Microbe Interactions

Process-based modeling and sensitivity analyses could identify which plant or microbial traits are most important in conferring crop resilience in different environments. Conceivably, these traits could then be engineered using synthetic biology to rationally and systematically design both plants and microbes (Farrar et al. 2014). Models can integrate multiple objectives—such as stress resilience, biomass yield, and soil organic matter formation—into design parameters. Because too many possible environmental conditions exist to test them all empirically, predicting the effects of interventions on crop resilience and performance relies on improved models (see Section 5.6: Modeling Opportunities and Needs, p. 60).

Chapter 4

Designing Resilient Cropping Systems

Along with maximizing plant performance and leveraging the microbiome, designing resilient feedstock cropping systems is an important strategy to increase crop resilience and productivity. Cropping system design encompasses crop choice, planting timing, and management practices. Introducing diversity in cultivars or crop species over spatial or time scales can confer resilience to the overall cropping system in addition to benefits attained from individual cultivars or species (see Fig. 4.1, p. 46). These strategies are currently both faster to deploy and farther advanced than crop improvement or microbiome engineering, creating nearer-term opportunities to enhance crop resilience at scale. Although outside the scope of this workshop, soil amendments (e.g., biochar and digestate from anaerobic digesters; see also Improve Soil Health, p. 40) were highlighted as another strategy to enhance resilient crop production by improving soil health.

4.1 Crop Diversity on a Spatial Scale

Various methods of increasing crop diversity on a spatial scale consider both genetic diversity and physical plant arrangements in the field (see Fig. 4.1A, p. 46). Plant–plant interactions, including resource competition, allelopathy, and indirect effects via microbial community changes, are a primary knowledge gap in need of further research to refine these methods.

Species Mixtures

Compared to monoculture crops, plantings of diverse crop species can exhibit improved yield, pest and disease control, and soil health (Iverson et al. 2014; Beillouin et al. 2021). These benefits may also reduce stand decline over time and improve the longevity of perennial feedstock cropping systems. However, the magnitude of these benefits depends on the planting density and species included in a crop mixture (e.g., legumes increase nutrient supply for all crops in the mixture; Iverson et al. 2014). Understanding the plant–plant interactions of different feedstock crop species will enable the design of cropping systems that take advantage of species complementarity to improve crop yield and resilience.

Polyclonal Plantings

Polyclonal plantings—genetically distinct varieties of the same crop species—could reduce the risk of monoculture susceptibility to abiotic and biotic stressors while maintaining overall productivity of the cropping system, as has been demonstrated for eucalyptus plantations in Brazil (Rezende et al. 2019). Planting clonal mixtures or different blocks of clones generally has positive effects on the productivity of short-rotation trees, such as willow and poplar, although it can depend on the clones included in the plantings (Dawson and McCracken 1995; DeBell and Harrington 1997). Complementary resilience against different stressors by different clones could ensure yield stability at the cropping system scale, but this has yet to be evaluated.

Intercropping

For row crops, intercropping feedstock crops with crops that provide disease suppression, nutrient provision, or

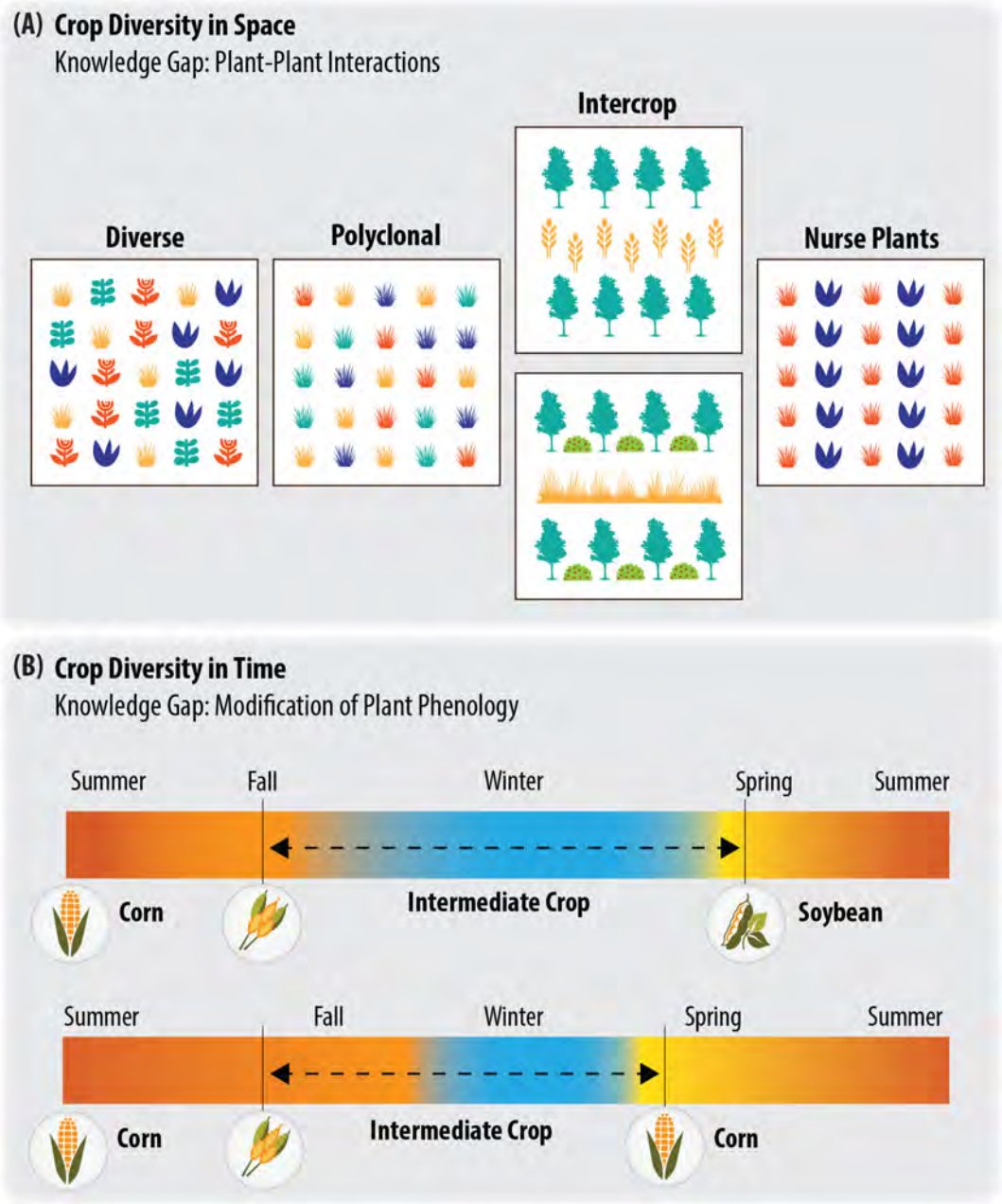


Fig. 4.1. Designing Diverse Cropping Systems. Resilient cropping systems designed to incorporate crop diversity over scales of space **(A)** or time **(B)** can increase yields for farm profitability, confer crop resilience at the cropping system scale, and improve soil health to sustain crop productivity over long-term cultivation. Methods of achieving diversity on a spatial scale include diverse species mixtures, polyclonal plantings, intercropping, and adding nurse plants. A key knowledge gap to widespread deployment is understanding the benefits and consequences of plant–plant interactions. Crop rotation and cover cropping are two methods of achieving crop diversity on a timescale. The harvest timing of intermediate crops in a cropping system affects the timing of summer cash crop planting. An earlier harvest, as illustrated by the leftward shift of the vertical line between winter and spring between the two timelines, enables planting of annual crops with a longer summer growing season. Shifts in planting timing may also minimize overlap between a crop's sensitive developmental stages (e.g., flowering) and commonly occurring stressors (e.g., summer drought). A key knowledge gap is modification of plant phenology (i.e., the different stages of a plant's life cycle) to optimize harvesting of mixed crops.

Key Research Opportunities To Improve Cropping System Design

- Improve fundamental understanding of plant–plant interactions to guide design of spatially diverse cropping systems for resilience.
- Modify plant phenology of intermediate crops to enable crop rotations or cover cropping compatible with primary cash crop planting and harvest schedule.

other soil health benefits can not only confer resilience to feedstock crops but also improve farm profitability by increasing overall productivity or introducing another cash crop. For example, despite reduced switchgrass productivity resulting from shading by intercropped poplar trees, overall biomass yield per land area increased in an intercropped system compared to a switchgrass monoculture (Kimura et al. 2018).

Research opportunities include determining which species intercrop well together and testing shade-tolerant crops and genotypes to intercrop with trees. For example, intercropping switchgrass with loblolly pines resulted in greater yields than monocultures of either crop; however, this was achieved only when a vegetation removal zone was implemented around the trees to reduce interspecific competition (Krapfl et al. 2017).

Nurse Plants

Nurse plants (i.e., companion plants) moderate harsh environmental conditions for young seedlings. Through this mechanism, they can facilitate the survival and growth of perennial feedstock crops under stress during their vulnerable establishment year (Gómez-Aparicio et al. 2008; Richwine et al. 2021), such as by suppressing weeds (Jungers et al. 2015). However, these benefits depend on species (Fagundes et al. 2018), plant size, and planting density (Anderson et al. 2016; Peláez et al. 2019).

Even if nurse plants do not confer benefits to perennial feedstock crop yields during establishment, they can produce harvestable biomass or grain as an additional income stream without detriment to biomass crop yields in subsequent harvest years (Jungers et al. 2015; Anderson et al. 2016). Thus, nurse plants can improve the economic resilience of feedstock cropping systems. Improved understanding of plant–plant interactions (in the context of choosing nurse plant species that confer benefits without competing with the main crop for resources) is needed to realize the potential of this resilient cropping system design.

4.2 Crop Diversity on a Timescale

Crop rotation and cover cropping are two means of achieving crop diversity on a timescale, with potential benefits for crop resilience and productivity (see Fig. 4.1B, p. 46). An overarching knowledge gap for this scale is how to modify the timing (i.e., phenology) of different stages (e.g., flowering) of an intermediate crop's life cycle to optimize its harvest and the subsequent planting of the summer cash crop.

Crop Rotation

Crop rotations, a practice in which different crops are planted sequentially on the same plot of land, can help maintain or improve soil health over time to support long-term resilient bioenergy crop production. Relay intercropping, in which a second crop is planted before the first crop is harvested, integrates spatial diversity into crop rotations as well. Oilseed crops and other emerging nonfood feedstock crops are new options for intermediate crops in a rotation that can provide an additional income stream to farmers and diversify the timing of feedstock supply to biorefineries.

Modifying the temperature sensitivity and flowering timing of intermediate crops to optimize planting and harvest timing for different latitudes will enhance productivity and allow farmers to better fit intermediate crops into existing rotations.

Cover Cropping

Cover cropping involves planting non-cash crops to improve soil health and control erosion and weeds. Research opportunities include designing cover cropping strategies based on crop lifespan. For example, cover cropping is viable for sugarcane but may not be suitable for perennial energy cane. In addition, because biomass sorghum is harvested green, the timing of sorghum harvest and cover crop planting can be optimized to balance dual goals of maximizing sorghum yield and achieving good cover crop establishment.

Chapter 5

Enabling Technologies, Shared Resources, and Infrastructure Needed for Resilient Crop Production

Innovations in enabling technologies, shared resources and infrastructure, modeling capabilities, and cross-disciplinary workforce training are needed to enable and accelerate breakthroughs in nonfood feedstock crop resilience research. While some needs are specific to plant or microbiome research, many are crosscutting. Common across all these needs is the potential for advances in AI/ML to speed data processing and interpretation; integrate multiscale data to identify key mechanisms underlying crop resilience and plant–microbe interactions; and predict plant and microbial performance across interacting genetic, environmental, and management contexts (G x E x M). However, before this potential can be realized, new enabling technologies must be developed to generate novel data needed to drive scientific discoveries.

5.1 Enabling Technologies Needed in Plant Genetics and Omics

Understanding and engineering plant resilience requires detailed insights into cellular processes, regulatory networks, and tissue-specific responses that operate across spatial, temporal, and developmental scales. Progress in this area depends on developing and deploying advanced tools, particularly for polyploid and nonmodel feedstock crops. Several priorities for innovating enabling resources and tools in plant genetics and omics were identified.

Advancing plant resilience research requires new enabling technologies to better link cell, tissue, and whole-plant responses to environmental conditions.

Single-Cell and Spatial Omics Analysis. Scientists widely agree on the value and importance of single-cell and spatially resolved transcriptomics, metabolomics, proteomics, and phenomics applications in resilient crop production. These methods are critical for understanding tissue- and cell-specific responses and resilience to stress, including the involvement of plant–microbe interactions. However, challenges in identifying cell- and process-specific markers, and scaling these technologies across nonmodel feedstock crops in both laboratory and field settings, must be addressed.

Tools for Functional Genomics and Polyploidy Studies. Enabling technologies needed for functional genomics include CRISPR-based genome-wide screens, mutant libraries, transformation vectors for gene stacking, and high-throughput validation systems to verify expected phenotypic outcomes. These tools are particularly lacking for complex polyploid genomes such as those of switchgrass, miscanthus, and willow.

Belowground, *in situ* phenotyping technologies that are high throughput and nondestructive are needed to promote understanding of how roots and rhizomes contribute to crop productivity and resilience.

High-priority areas include understanding why current polyploids behave differently from diploids and how synthetic hybrids may help unlock genetic potential.

Multiomics Data Integration and Analysis. Robust analysis of complex omics datasets—including transcriptomic, metabolomic, proteomic, and phenomic data—requires interoperable databases, standardized metadata, and user-friendly bioinformatics pipelines. Developing such tools and leveraging AI/ML for integrative analysis of gene networks across species, tissues, and treatments are essential, particularly for predictive modeling of phenotype from genotype.

Advanced Transformation and Gene Editing Platforms. Continued improvements of technologies like *Rhizobium rhizogenes*–based transformation, protoplast systems, and viral delivery systems for *in vivo* gene editing are necessary to accelerate gene functional validation. Additionally, developing plant-centric robotic systems and microfluidics platforms will allow high-throughput screening of gene interactions and significantly increase the pace of discovery. A previous DOE report, *Overcoming Barriers in Plant Transformation: A Focus on Bioenergy Crops*, details needs and opportunities in bioenergy crop transformation (U.S. DOE 2024b).

Biosensors, Real-Time Sensing Technologies, and Phenotyping Platforms. Advances in biosensors capable of detecting different molecules, metabolic processes, and physiological parameters are critical to enhancing *in vivo* assessments of the spatiotemporal dynamics of plant stress responses. Additionally, noninvasive, field-deployable sensors—especially those integrated with imaging or remote-sensing technologies—are needed to link genotype to responses to environmental conditions in real time. Continued development of high-throughput phenotyping platforms for field and controlled environments (see Fig. 5.1, p. 51), single-cell screening devices, and robotic systems for gene function validation will allow rapid scaling of experiments. These tools should enable multimodal analysis across plant development stages and stress environments.

Belowground Phenotyping Technologies. Belowground sensors are needed to advance understanding of how root systems and their interactions with soil and microbes contribute to crop resilience. Traditional methods often require separating roots from soils, which fundamentally destroys the physical environment in which belowground processes occur. Root and rhizome growth can also be spatially patchy. Therefore, ground-penetrating technologies, in-ground rhizotron cameras, and other sensors that can be deployed at relatively high spatial resolution are critically needed for studying roots and rhizomes *in situ*. Soil and plant sensor arrays that synchronously measure plant phenotypes across growth cycles and environmental conditions will elucidate how plant processes change as root and rhizome systems develop and soil conditions change.

5.2 Shared Resources Needed To Accelerate Plant Research

Advancing feedstock crop research and development requires access to well-curated genetic and genomic resources spanning a wide range of species and genotypes. These foundational resources are critical for efforts to link genotype to phenotype and will support reproducible experimentation and predictive modeling of complex traits. Funding for partnerships between public entities and private industry that encourage sharing of knowledge, tools, and germplasm could greatly expand the breadth of resources available to researchers. New experimental approaches and avenues are also needed to test hypotheses about fundamental physiological mechanisms and plant performance across spatial and temporal scales in diverse environments. Several high-priority shared tools and resources are needed to achieve these goals.

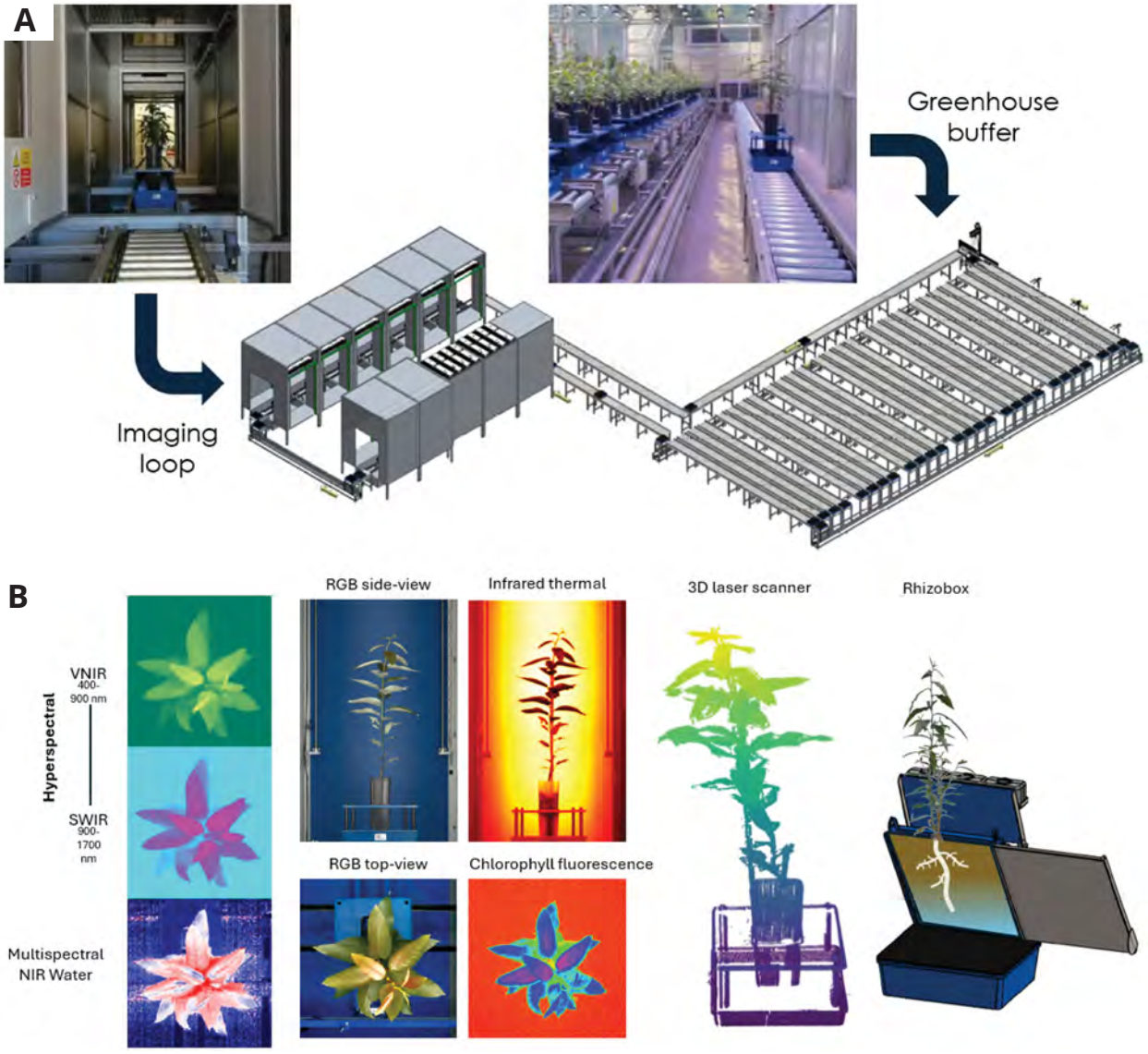


Fig. 5.1. High-Throughput Phenotyping. The Advanced Plant Phenotyping Laboratory (APPL) at Oak Ridge National Laboratory leverages a robotic conveyor system to grow plants in various pot configurations and rhizoboxes. **(A)** In a controlled greenhouse, the conveyor system moves plants through a weighing and watering station and an imaging loop with advanced cameras covering a broad spectrum of light. It then automatically returns the plants to their original positions. The system accommodates 520 tray positions, with the potential to screen 10,000 *Arabidopsis* plants or hundreds of poplar trees. **(B)** APPL has among the most diverse imaging arrays for plant measurements in the world, with a total of 11 modalities. These include color (RGB, red-green-blue) and thermal imaging; chlorophyll fluorescence; near infrared (NIR) multispectral, visible-near infrared (VNIR) hyperspectral, and short-wave infrared (SWIR) hyperspectral imaging; 3D laser scanning; and a new belowground upgrade with color root imaging and NIR soil water imaging. These sophisticated methods enable nondestructive assessment of whole-plant development and stress responses over time. [Courtesy Oak Ridge National Laboratory]

Fig. 5.2. *Miscanthus sacchariflorus* Diversity Panel. The panel, established at the University of Illinois Energy Farm in Urbana, Ill., contains more than 200 *M. sacchariflorus* accessions (i.e., a uniquely identified sample of plant material collected from a particular area), some from parts of the world no longer accessible for future collections. Grouped into four replications, each framed within border rows of *Miscanthus x giganteus* "Illinois," the panel captures diverse adaptation across a broad geographic range, including genetic variation in winter hardiness that is critical for breeding or engineering improved cold tolerance in miscanthus. The panel represents an irreplaceable genetic resource for the U.S. bioeconomy. [Reprinted under a Creative Commons Attribution 4.0 International license (CC BY 4.0) from Njuguna, J. N., et al. 2023. "Biomass Yield in a Genetically Diverse *Miscanthus sacchariflorus* Germplasm Panel Phenotyped at Five Locations in Asia, North America, and Europe," *Global Change Biology Bioenergy* **15**(5), 642–62, 258. DOI:10.1111/gcbb.13043.]

Shared, well-curated, and supported genetic and genomic resources that enable reproducible, cross-scale studies will accelerate crop research for the bioeconomy.

Public Genetic and Genomic Resources. A critical need exists for gene atlases, sequence-indexed mutant collections (including gain-of-function lines), and core germplasm sets with corresponding omics data. These genetic and genomic resources underpin the ability to dissect genetic, molecular, and physiological mechanisms and leverage them to enhance plant stress tolerance. These resources must include both model and nonmodel species and reflect diverse genetic backgrounds. Given the major investment needed to develop genetic and genomic resources, some of which are irreplaceable (see Fig. 5.2, this page), long-term plans for their maintenance and preservation are imperative.

Pangenomes and Reference Databases. Availability of whole-genome sequence information is critical to advancing crop research, including accelerating genetic improvement and developing mechanistic understanding of stress resilience processes. Increasing the size and resolution of pangenomes to capture family- and species-specific variation is essential. Curated, centralized databases, similar to The Arabidopsis Information Resource (arabidopsis.org) but for feedstock crops, should integrate genomic, transcriptomic, phenomic, and expression data in a publicly accessible format.

Transformation Facilities and Platforms. New insights from genetic and omics studies will identify avenues to improve the stress resilience of feedstock crops. To fully leverage these insights and test increasing numbers of gene targets, facilities are needed that support plant transformation, particularly genotype-specific systems for crops with limited transformability. These facilities should be regionally distributed to accommodate regulatory and biosafety constraints and should offer workforce training for broader community access.

Repositories and Biobanks. Repositories for genomic plant DNA, RNA, proteins, and plant-associated microbial strains (e.g., mycorrhizal fungi and endophytes) must be expanded and standardized. Properly archived biological materials, along with protocols for sample collection and processing, will enhance reproducibility and collaboration across research groups.

5.3 Enabling Technologies and Resources Needed in Plant Microbiome Research

Research on plant–microbiome interactions is rapidly evolving, yet technical, methodological, and conceptual challenges must be overcome to successfully manage crop microbiomes for resilient production. While some researchers are optimistic that single engineered microbial strains can be modified to benefit the host plant, another successful approach may include managing the functions of obligate microbial symbionts (e.g., arbuscular mycorrhizal fungi) or whole consortia. New research should focus on decoding microbiome functionality, quantifying active interactions between plants and their microbiomes, and identifying key microbial traits that can be managed to improve plant resilience. New technologies and resources are needed to coordinate sampling of these multiple data streams and organize and share data.

Tools for Identifying Key Taxa and Functions. Plant microbiome studies need to move beyond characterization of "who's there" toward a more function-oriented perspective. Doing so will require techniques focused on active microbial taxa, such as quantitative stable isotope probing (qSIP; Hungate et al. 2015), single-cell activity (Lindsay et al. 2025), quantitative transcriptomics (Gifford et al. 2014), and new approaches for exometabolite annotation and gene interpretation (Erbilgin et al. 2019). Fabricated ecosystems for whole-plant isotope tracing enable tracking of carbon assimilated by plants into active microbes and soil organic matter under controlled conditions (see Fig. 5.3, p. 54; Pett-Ridge and Firestone 2017; Pett-Ridge et al. 2020); however, these systems often are not large enough to track high-yielding grasses to maturity. Advances in functional imaging of plant–microbe interactions are also needed, particularly for the endosphere and rhizosphere (Lee et al. 2022). Due to the dominance of plant DNA in endosphere and rhizosphere samples, new approaches are needed to deplete plant DNA and improve methods to analyze the combined plant–microbe metagenome and metatranscriptome.

Leveraging plant microbiomes to improve feedstock crops will require resources that elucidate microbial function and traits.

Improved Sequencing Methods, Trait Identification, and Data Tools. Current microbiome sequencing involves trade-offs in throughput, cost, and data inference. Genome-resolved metagenomics can infer functional potential, but for complex systems, sequence assembly is a lengthy and computationally expensive process (Riley et al. 2023) that resolves a limited number of taxa (e.g., fungal and eukaryotic genomes assemble poorly). Amplicon-based approaches, while affordable, provide less functional information. New informatics approaches are needed to bridge this gap, including better genome curation, techniques linking marker gene sequences to function (Douglas et al. 2020), and orthologies designed specifically for plant–microbe signaling and recruitment mechanisms. AI may be better able to link marker gene information from metagenome-assembled genomes with information from 16S ribosomal RNA amplicon sequencing. For all approaches, combining long-read sequencing and qSIP can better link function, phylogeny, and activity.

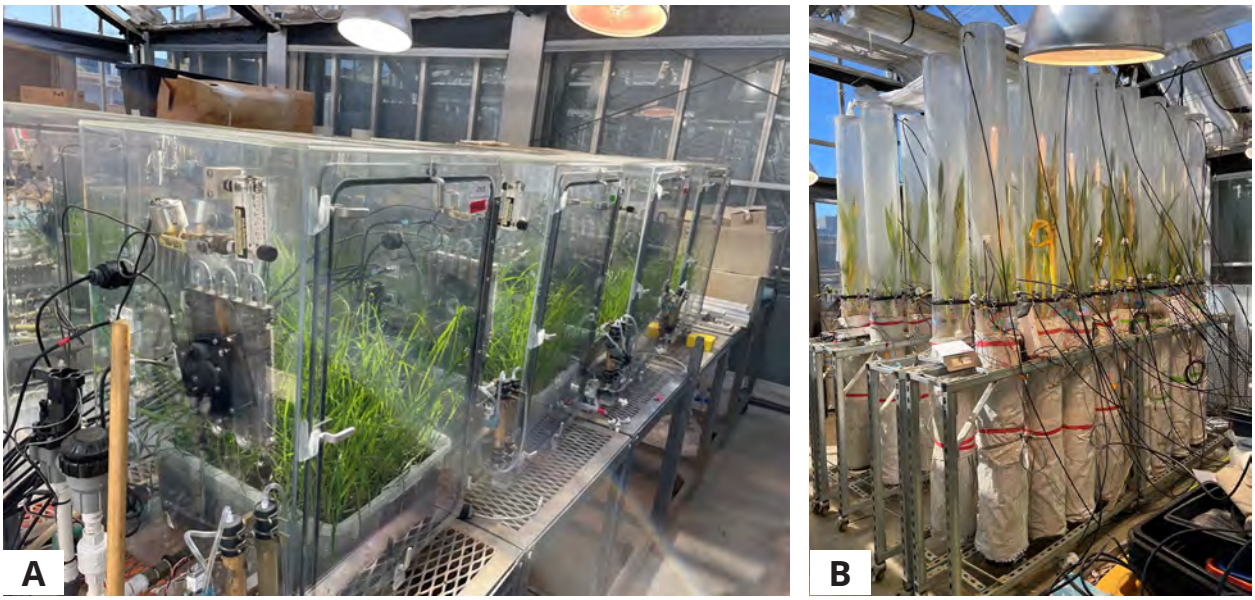


Fig. 5.3. Whole-Plant Isotope Tracing for Microbial Function Studies. (A) Wild oat (*Avena barbata*) and oil sorghum (*Sorghum bicolor*, a transgenic elevated oil line produced by the Center for Advanced Bioenergy and Bioproducts Innovation; Park et al. 2024) are grown at the Environmental Plant Isotope Chamber Facility in Berkeley, Calif. This fabricated ecosystem facility supports replicated whole-plant carbon-13-labeled carbon dioxide ($^{13}CO_2$) isotope tracing studies under controlled light, humidity, and temperature conditions. Carbon may be quantitatively tracked from the atmosphere into plant tissues, root deposits, soil microorganisms, and mineral associations. **(B)** A custom sensor relay system monitors and dynamically adjusts headspace CO_2 and ^{13}C isotope concentration. [Courtesy Lawrence Livermore National Laboratory]

Tools for Identifying Key Microbial Traits. New approaches are needed to identify the plant traits that mediate microbiome assembly and whether they are steady or variable over time. Similarly, the microbiome traits that are most essential for resilience should be identified, such as those that enhance nutrient uptake in crops under stress. New pipeline tools like *microTrait* (Karaoz and Brodie 2022), FUNGuild (Nguyen et al. 2015), DRAM (Distilled and Refined Annotation of Metabolism; Shaffer et al. 2020), and FAPROTAX (Functional Annotation of Prokaryotic Taxa; Louca et al. 2016) may be able to provide this information by resolving microbial functional traits from sequence data.

Gene Editing and Biosecurity for Beneficial Microbe Studies in the Field. Novel genetic tools, such as CRISPR-Cas9 gene editing, can be used to engineer rhizosphere microorganisms and identify and validate the key mechanisms by which microbes affect crop performance (Garcia et al. 2013; Song et al. 2019). Improving the effectiveness and predictability of beneficial microbes after they are introduced into field settings is critical for realizing the potential benefits of microbiome engineering. Biocontainment strategies and biomonitoring are essential biosecurity measures to develop for engineered microbes introduced in the field.

Novel Methodologies for Measuring *In Situ* Plant–Microbe Interactions and Biogeochemical Processes. Benefits from managed plant–microbiome interactions must be measurable at the field scale. Thus, field-based manipulation facilities are essential to evaluating how individual and combined environmental stressors affect plant–microbiome interactions and translate to biogeochemical effects. These facilities should continuously collect data with sensor arrays that collect both microbial and biogeochemical information across growth cycles and variable conditions. Tools for continuous measurements of isotope tracers and gross process fluxes (e.g., gross, instead of net, nitrogen mineralization rates) should be developed.

Microbiome Data Sharing, Resources, and Standardization. Sampling should be coordinated across

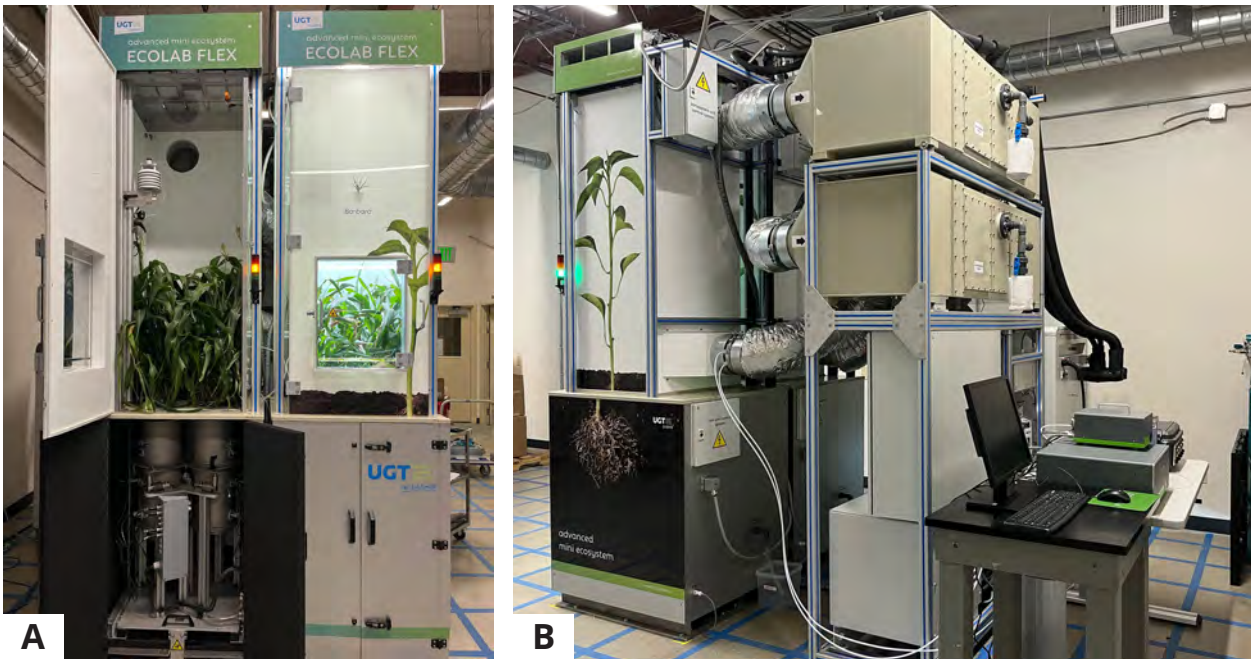


Fig. 5.4. Laboratory Ecosystems. EcoPOD growth chambers at Lawrence Berkeley National Laboratory precisely control climatic conditions to simulate field environments and support plant growth and monitoring throughout the entire life cycle **(A:** two identical units sit side-by-side with doors open on the left chamber). EcoPODs are equipped with a bottom soil unit containing stainless steel lysimeters (visible inside open black cabinet) with sampling ports, soil moisture and temperature regulators, and real-time monitoring sensors. An aboveground growth chamber **(A:** see plants visible inside) provides programmable LED lighting and humidity and temperature control. The growth chamber is connected to a gas analyzer **(B:** see gray boxes on table next to computer) for continuous monitoring of gas fluxes during plant growth. [Courtesy Lawrence Berkeley National Laboratory]

disciplines by collecting plant, ecosystem, and microbial data simultaneously. Better organization and availability of data (including metadata) and improved annotation tools will help achieve FAIR data standards (Findable, Accessible, Interoperable and Reusable; Wilkinson et al. 2016). National efforts like the National Microbiome Data Collaborative are working toward this goal by standardizing metadata and expectations for data sharing. Similarly, overt efforts should be made to share resources (e.g., isolate collections).

5.4 Research Infrastructure and Cyberinfrastructure Opportunities and Needs

Critical research infrastructure needs include long-term, shared facilities for laboratory, greenhouse, and field experiments instrumented with high-throughput, automated, above- and belowground phenotyping systems. The facilities should enable precise control over multiple conditions simultaneously to test how individual and combined environmental stressors alter plant, microbe, and soil processes and their interactions. In parallel with research infrastructure development, improved cyberinfrastructure capabilities are needed to manage and analyze the large data streams generated in these facilities.

Research Infrastructure

Laboratory Facilities. Controlled laboratory facilities help bridge the gap between reductionist laboratory studies and complex field environments, enabling scientists to quantify processes that are difficult or impossible to accurately measure under field conditions. Laboratory ecosystems, such as the EcoPOD (see Fig. 5.4, this page), enable experiments under highly

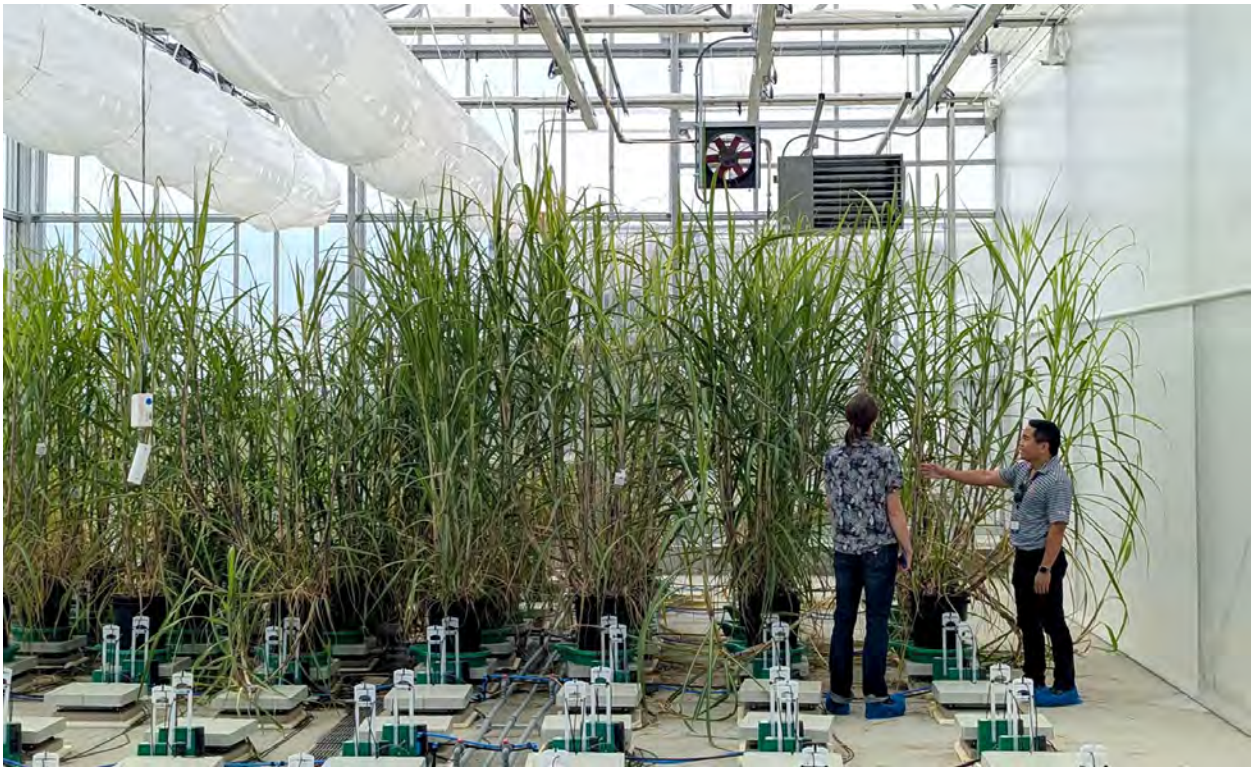


Fig. 5.5. Tall Greenhouse Facilities Can Accommodate Phenotyping of Large Bioenergy Grasses Like Sugarcane. The Plant Biology Innovation Greenhouse at the University of Illinois Urbana-Champaign was designed to accommodate high-yielding bioenergy grasses. It is outfitted with an automated lysimeter system for monitoring individual plant growth and water use, enabling high-throughput phenotyping of water-use efficiency. [Courtesy University of Illinois Urbana-Champaign]

Complementary laboratory, greenhouse, and field facilities enable research linking plant, microbial, and soil processes across scales and environments.

controlled yet environmentally realistic conditions while maintaining the reproducibility necessary for mechanistic discoveries. Equipping these facilities with diverse sensors for real-time, quantitative, nondestructive measurements—coupled with complementary imaging capabilities—is necessary to connect plant form and function to environmental conditions.

Greenhouse Facilities. Greenhouses built taller than standard size are needed to accommodate tall feedstock crops, including perennial grasses and trees (see Fig. 5.5, this page). Such greenhouses should provide controlled conditions to simulate exposure to drought, heat, flooding, elevated carbon dioxide, nutrient limitations, and stressor combinations. Biosafety controls and compartmentalization can enable studies of pathogens in addition to abiotic stressors by preventing cross-contamination between inoculated and uninoculated plants. For studies involving microbial inoculation, digital droplet polymerase chain reaction (ddPCR) equipment should be included to correlate plant phenotypes with quantification of strain-specific microbial population.

Field Facilities. Manipulation experiments at field facilities, as well as large networks of common gardens across environmental gradients, can provide better understanding of the interaction effects between genotype and environment (G x E) on plant, microbe, and

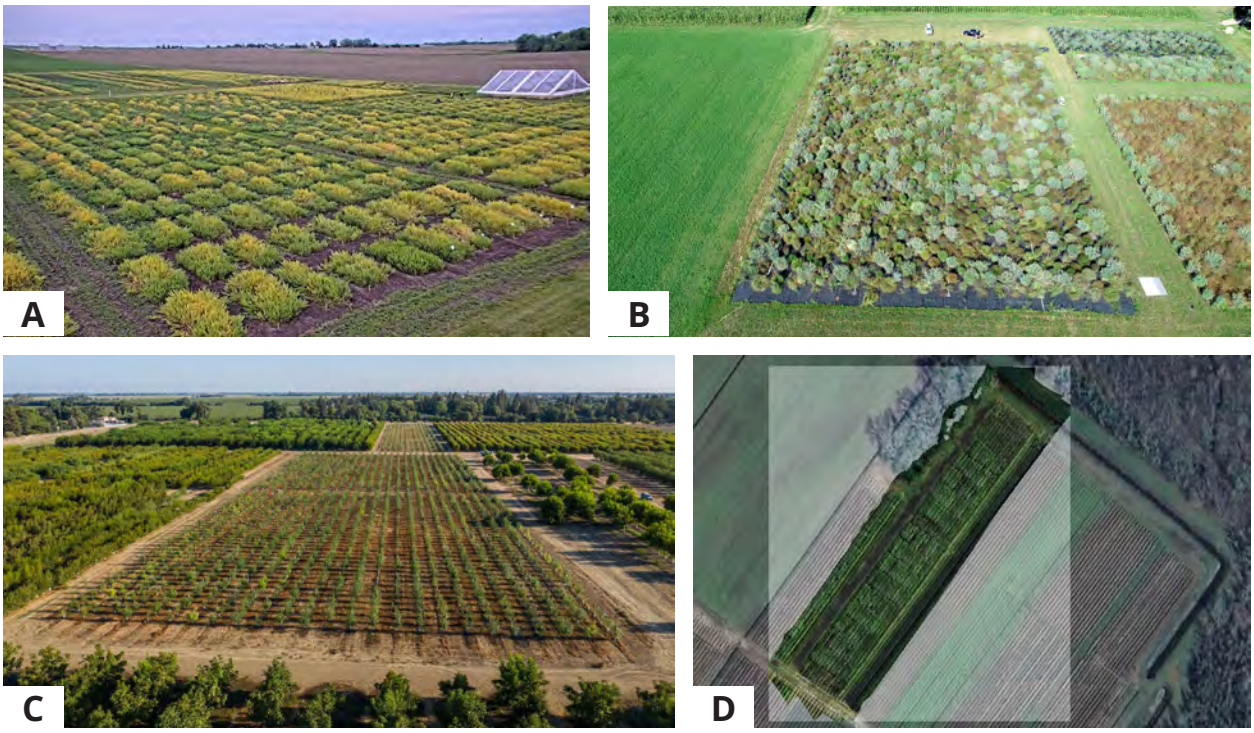


Fig. 5.6. Field Experiments Conducted Across a Broad Range of Environments Are Critical to Evaluating Feedstock Crop Genotypes and Their Interactions with the Environment. (A) Pennycress common garden plots near Macomb, Ill., planted with natural accessions collected from environments around the world, contain large genetic diversity, as indicated by differences in spring maturities. A rainout shelter (upper right) excludes rain for drought experiments. **(B)** A switchgrass diversity panel established at the Kellogg Biological Station in Michigan is part of a multilocation common garden study examining the genetic basis of adaptation to different environments, stress tolerance, and biomass production. **(C)** A *Populus trichocarpa* diversity panel was established at the University of California–Davis to elucidate the genetic basis of drought tolerance and yield. **(D)** A mosaic image of an energy cane yield trial shows diverse germplasm in the fifth year after planting. Multispectral drone images were taken every two weeks during the growing season to monitor crop growth and development, then overlaid on satellite photographs of the field. The experiment was located at the U.S. Department of Agriculture–Agricultural Research Service's Sugarcane Research Unit field site in Houma, La. [(A) Courtesy Winthrop Phippen, Western Illinois University; (B) Courtesy Michigan State University; (C) Courtesy Gail Taylor, University of California–Davis; (D) Courtesy University of Illinois Urbana-Champaign]

soil processes (see Fig. 5.6, this page). Common gardens should be instrumented with microscale weather stations—to evaluate environmental conditions with high spatial resolution throughout the site—and monitored by regular drone and satellite imaging modalities (see Fig. 5.7, p. 58). Baseline measurements of soil properties, hydrology, and microbial community composition at field sites would help researchers interpret site data.

Crosscutting Infrastructure Needs. All facility types could benefit from high-throughput, automated sensors generating time-series data for determining causal

Understanding resilience requires comprehensive crop performance datasets in replicated common gardens at scale, across environmental gradients, under abiotic stress, and on marginal soils.

Fig. 5.7. Common Garden Networks Encompass Diverse Environmental Conditions and Are Equipped with Advanced Sensing Modalities. Standardized measurements of plant, soil, microbe, and environmental conditions come from an integrated set of sensors, drones, weather instruments, and satellite imaging.

relationships in plant, microbial, and soil responses to stressors (see Section 5.5: Instrumentation Opportunities and Needs, p. 59). In addition, high-resolution phenotyping across scales—from cells to tissues, organs, and plant canopies—would yield invaluable insights into mechanisms regulating plant productivity and tissue composition. Some advanced sensor equipment may be prohibitively expensive and may require highly trained personnel, so making it centralized and shared provides a broader community of researchers with access to both the equipment and generated data.

Cyberinfrastructure

To fully leverage controlled environment and field facilities and networks, developing cyberinfrastructure capabilities is essential. For example, cyberinfrastructure supporting more robust and broadly applicable plant phenotyping could speed generation of basic knowledge about the mechanisms underlying crop resilience while creating datasets that can be leveraged for AI/ML applications (U.S. DOE 2023).

At the field level, drones, satellites, and sensors generate data that, if integrated into high-throughput data pipelines (e.g., Varela et al. 2021; Rajurkar et al. 2022), can connect and correlate crop performance with genetic traits, the plant microbiome, soil amendments, and other agronomic practices. In some cases, this data integration requires developing open-source software packages, such as PlantCV (plantcv.org). Such platforms may often be used off-the-shelf for plots across

many different sites, although some customization is typically required. Democratizing the use of these software packages can broaden the use and impact of rapid plant phenotyping approaches without requiring extensive experience.

Leveraging drone imagery and other high-throughput sensor data also requires scalable cloud storage that collaborators across multiple institutions can easily use to perform complex analyses and access data via application programming interfaces (API). Integrating 5G devices, networks, and applications into superfacility workflows adds complexity and calls for innovative solutions to integrate distributed wired and wireless instruments, data, and computing facilities over high-performance networks.

5.5 Instrumentation Opportunities and Needs

New instrumentation can enable research breakthroughs and significantly accelerate the pace of discovery. Innovation in new sensors and instrumentation for plant phenotyping across biological scales—from the subcellular to the population level—will require close collaborations between biologists and engineers. These novel tools for high-resolution form and function plant phenotyping are needed (1) to study the mechanisms of plant and microbiome responses to stress in real time and (2) to investigate how improving plant genetics, manipulating the microbiome, and changing crop management practices alter these responses. Most importantly, instrumentation and associated computational tools that increase data collection and processing throughput are needed to measure plant stress responses throughout development and track interactions among genetics, environment, and management relevant to crop resilience.

New Instrumentation

A new generation of tools capable of capturing biological complexity across spatial, temporal, and organizational scales is needed to advance crop resilience research. Priority instrumentation needs include imaging, sensing, and analytical technologies.

Robust cyberinfrastructure development should accompany establishment of shared research facilities to collect, manage, and integrate sensor data and accelerate discovery of resilience mechanisms.

Plant Imaging Across Scales. To better understand plant cellular responses to abiotic stressors and how they alter plant–microbe interactions at the mechanistic level, imaging technologies that capture dynamics at the intracellular to tissue levels are necessary.

Improved Metabolomics. Innovations are needed to more efficiently characterize the many unknown metabolites and proteins from beneficial microbes and in plants responding to abiotic and biotic stressors. Instruments that expand the number of measurable and identifiable metabolites, such as those employing increased sensitivity of nuclear magnetic resonance output, are needed to improve metabolomics research, as are integrated experimental and informatic analyses of mass spectrometry outputs for robust annotation.

Nondestructive Belowground Sensors. Noninvasive, nondestructive instruments for high-throughput evaluation of root systems and their interactions with the biological, chemical, and physical properties of soil in the field are needed to transform understanding of belowground processes that contribute to crop resilience and soil health. Moving beyond traditional red-green-blue imaging to other modalities (e.g., hyperspectral and ultraviolet imaging) in minirhizotron-based approaches—currently used for measuring root length *in situ*—could reveal additional features of roots and the soil environment. Other nondestructive measurements of root and soil characteristics may also hold promise, including those attempted in both controlled settings (e.g., X-ray, neutron imaging, and magnetic resonance imaging) and in the field (e.g., ground-penetrating radar and electrical capacitance).

Large-Scale Microbial Inoculation Technology. For large-scale testing of diverse microbial inoculants,

equipment and methods must be developed to grow large inoculant volumes, preserve inoculant viability, and precisely apply microbes in the field.

Plant Microbiome DNA Extraction. Instrumentation is needed to analyze the microbiome of large feedstock crops. DNA is normally extracted from a subset of tissues, and the microbiome data is then extrapolated to the whole plant. However, this approach is inaccurate for large plants because the microbiome can vary by plant organ, location within the canopy, and location along the root system.

High-Precision Harvesters. The impacts of abiotic stressors on crop yields are a major indicator of crop resilience. However, existing harvesters are not designed to measure biomass yield from bioenergy grasses. High-precision harvesters like those developed for food crops need to be engineered for feedstock crops to measure yields quickly and accurately, with high spatial resolution in large research plots and commercial fields.

Innovations in sensors and instrumentation are essential to accelerate fundamental and applied research from molecular to field scales.

Novel Integration of Instrumentation

Progress in improving crop performance across diverse environments and stressors hinges on understanding fundamental mechanisms and the interrelatedness and hierarchies of stress response processes across all levels of biological organization, from the molecular to the organismal and field scale. To better study mechanisms and their interrelatedness, novel instruments and integrating methods are critical, including:

Evaluations of Plant Traits and Physiological Processes. Existing instrumentation (e.g., thermal, hyperspectral, and lidar-based sensors), increasingly applied at the organismal level in controlled and field conditions, needs to be complemented with instrumentation that can assess physiological processes (e.g., gas exchange in photosynthesis and respiration) and nutrient and hydraulic fluxes in a high-throughput manner. For example, robotics for precise simultaneous trait evaluation (e.g., stress detection and growth parameters) and direct high-throughput measurements of physiological processes (e.g., photosynthesis) would provide advantages over current methods, including improved efficiency, relevancy, and deeper characterization.

Measurements of Root Exudates and Microbial Responses. To better understand interactions between plants and the rhizosphere microbiome, complementary sensors are needed that can (1) determine root exudation quantity and composition in space and time and (2) provide information about microbiome activity and composition under field conditions in real time.

High-Throughput Plant Phenotyping Across Geographic Scales. Drone-mounted sensors are already revolutionizing high-throughput plant phenotyping at the canopy and field scale, but the full potential of remote sensing for phenotyping is still unrealized. Integrating ground-based and proximal remote-sensing methods with satellite imagery that provides high spatial and temporal resolution would enable large-scale, uniform phenotyping across multistate field trials and over the course of multiple years. Integrating real-time data processing capabilities would further increase throughput to accelerate research progress and, if sensors become affordable for broad use, enable rapid decision-making by farmers. However, extensive ground-truth data is needed to validate sensor-based phenotyping. Developing new methods to collect this data can address the context dependency of AI/ML approaches for data processing and data fusion and overcome their need for large amounts of training data (e.g., Varela et al. 2025).

5.6 Modeling Opportunities and Needs

Crop production results from a complex web of interactions among plants, microbes, soil processes, and environmental conditions that makes it challenging to predict how crop productivity and composition will respond to stressors. Predictive AI/ML approaches

stand to accelerate the development and testing of resilient cropping systems. For example, AI/ML can help select genes for plant resilience traits, beneficial microbes, and soil amendments that are best suited to different soil types and growing regions. However, such approaches are currently hampered by a lack of well-replicated mechanistic data and fundamental understanding of plant and microbial processes and plant–microbe interactions needed to build robust, predictive models. While sources like the U.S. Department of Agriculture (USDA) and NASA share ample data, integrating understanding of fundamental mechanisms with these datasets into models will require collaboration between domain experts and computer scientists.

Most models that explicitly incorporate microbes are still in early development and cannot yet address key research questions. No models are currently capable of identifying region-specific plant microbiome traits benefiting crop performance, so the biological solutions (i.e., microbial inoculants) required to address limitations in specific locations cannot yet be predicted. Ultimately, software tools should be developed for pairing cultivars, microbial amendments, soil amendments, soil types, and environmental conditions with optimal agronomic practices. Realizing this potential will require advanced computing and sharing high-quality collaborative data.

Model Capabilities

Off-the-shelf algorithms are often an adequate first step in integrating AI/ML approaches into cropping system models. However, the boundaries of integration efforts must be pushed to develop new algorithms tailored to the needs of the feedstock crop research community. Several high-priority needs in developing new model capabilities were identified.

Integrating Microbial Processes. The highest priority need is to develop microbially explicit models that represent the role of microbial processes in ecosystem models. Plant–microbe interactions are important not only because they directly benefit or harm plant survival and growth but also because they exert indirect effects by mediating soil processes that contribute to plant and soil health. However, few microbially explicit models currently exist and all are still in early development [e.g., FUN-BioCROP (Fixation and Uptake of Nitrogen-Bioenergy Carbon, Rhizosphere, Organisms, and Protection; Juice et al. 2022); DayCent-CABBI (Center for Advanced Bioenergy and Bioproducts Innovation; Berardi et al. 2024); MEMCN (Microbial-Explicit Model incorporating Comprehensive Nitrogen processes; Yan et al. 2024)].

Developing predictive, region-specific feedstock cropping systems will require integrating mechanistic understanding with advanced AI/ML models.

These models represent microbial metabolism using Michaelis-Menten kinetics that characterize broad microbial functional groups. A critical need exists to develop a tool that can predict microbial function from phylogeny, thereby enabling microbial omics data to inform models. Tools to link microbial omics data to biogeochemical parameters and plant physiological parameters are also needed to model connections among plants, microbes, and soil processes.

Linking Models. Linking techno-economic analysis (TEA), life cycle assessment (LCA), economic models, and Earth system models is critically needed to predict feedstock crop production and soil processes under future environmental conditions and under different possible policy and market scenarios. These models, where possible, should be calibrated and validated with empirical data. Furthermore, creating common data structures and data sharing platforms can increase transparency and interoperability.

Major challenges in linking these models include different spatial and temporal scales, model input components, and model input definitions. Leading agroecosystem models, such as DayCent (Berardi et al. 2024), have a limited ability to represent phenotypic modifications in plants, such as changes in root exudates or cell wall composition. In addition, models

Datasets spanning genetics, physiology, microbes, soils, and environments are needed to support model development and reduce uncertainty in model predictions.

predicting location-specific rainfall and temperature must be downscaled to produce results relevant to particular growing regions and seasons. However, high granularity is not always necessary. Reduced-order modeling approaches are useful when simplified representations of particular systems are adequate to answer central research questions.

Efficient AI/ML Model Training. AI/ML has the potential to untangle complex G x E x M interactions, but the approach carries high computational costs and requires vast datasets. Generating such datasets can be expensive and time-consuming, particularly when long-term replicated field trials are required. Reducing these burdens requires innovating AI/ML approaches to develop less context-dependent and more efficient model training (e.g., few-shot models). For example, using matched messenger RNA and trait data to reduce ML dimensions can improve gene-to-trait predictive power and identify gene sets that predict phenotypes (e.g., Cheng et al. 2021).

Blending Mechanistic and AI Models. Because it is impossible to generate field data in all G x E x M contexts, innovations in blending mechanistic models with AI models are needed to overcome the massive amounts of data required for AI foundation models. For example, knowledge-guided ML can leverage mechanistic understanding from process-based models to reduce uncertainty in model predictions when empirical data are limited (e.g., Liu et al. 2024).

Surrogate Models. AI/ML can also be used to create surrogate models with improved computational efficiency for use in place of complex, large-scale models, thus enabling a broad base of users to vary the subset of conditions most relevant to their applications. Surrogate models that do not require supercomputers increase user access to modeling capabilities needed to accelerate research and development. For example, surrogate models can enable modelers and empiricists to more easily and rapidly test model sensitivity and behavior. This process identifies the parameters that are most important for improving mechanistic understanding of the modeled processes and for generating data that reduce model uncertainty.

Data To Support Model Innovations

Data and mechanistic understanding are the foundation of good predictive models. DOE has a unique advantage in model training because of its ability to support large collaborative efforts that can generate large AI-ready datasets. Thus, DOE has an opportunity to lead by example and release datasets for use in AI models that benefit the entire scientific community. Several high-priority data needs were identified for reducing uncertainty in model predictions and supporting innovations in model capabilities to accelerate progress toward resilient feedstock crop production.

Crop Data from Diverse Environments. Physiological and genetic data collected from diverse germplasm grown in multilocation field trials that capture G x E x M interactions will increase both the breadth and resolution of inference space in model predictions (see Fig. 5.8, p. 63). Increasing inference space will reduce model uncertainty and enable scientists (1) to gain predictive understanding of the best genotypes for specific sites with predicted environmental conditions and (2) to identify the opportunity space for nonfood feedstock crops to outperform conventional crops. Field trials across environmental gradients should include multistressor environments to understand how plant, microbe, and soil processes and their interactions influence how crops react to stressors in the real world. For example, common garden studies across environments are critical for obtaining sets of complementary data (e.g., genetic, metabolomic, transcriptomic, physiological, and phenotypic data) for both plants and microbes to determine how each

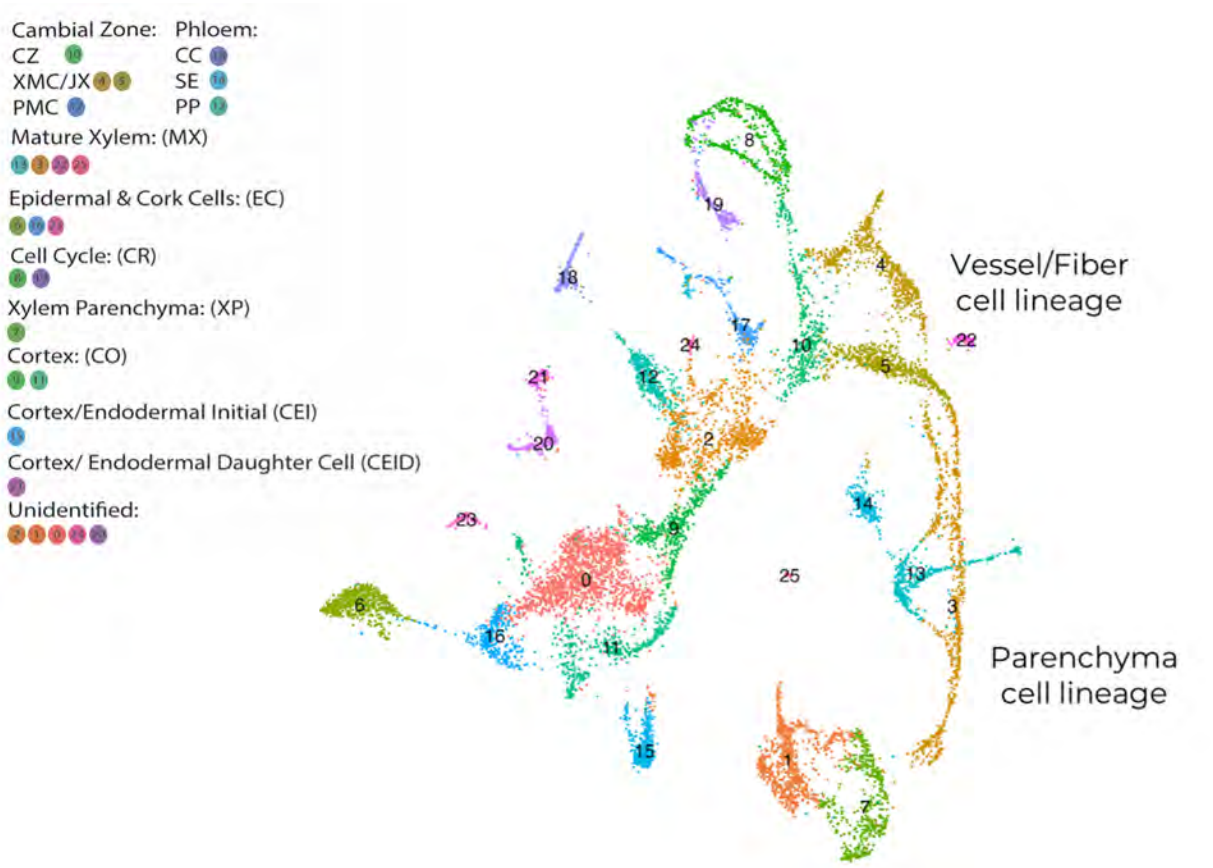


Fig. 5.8. Availability of Complementary Data Across Biological Scales of Organization Will Increase Inference Space in Model Predictions. Omics data (e.g., single-cell transcriptome analyses) provide valuable information about the function of specific cell types and relationships among cell types. For example, RNA sequencing of nuclei isolated from a *Populus* stem identified 26 gene expression clusters (numbered 0 to 25) representing different cell types. Using trajectory analysis, researchers identified vessel, fiber, and parenchyma cell lineages that they then used to discover transcripts that regulate the differentiation of meristematic cells into each cellular component of wood biomass. [Reprinted under a Creative Commons-Attribution-NonCommercial-No Derivatives 4.0 International license (CC BY-NC-ND 4.0) from Schmidt, H. W., et al. 2025. "Deep Tissue Profiling of *Populus* Stem at Single Nucleus Level Reveals Uncharacterized Cell Types and Cell-Specific Gene Regulatory Networks," *Genome Biology* **26**(1), 258. DOI:10.1186/s13059-025-03728-x]

changes under different scenarios and impacts resilient crop production.

High-Throughput Field Data. High-throughput field and molecular data from different feedstock crops, including biodesigned lines and genetically diverse germplasm, are needed to train AI/ML models. Model training gains statistical power for deciphering fundamental mechanisms and discovering stressor- and yield-related genetic variants, both natural and induced. High-throughput data from remote-sensing technologies and forthcoming in-ground sensor networks will be essential for above- and belowground phenotyping.

Omics and Functional Data on Plant–Microbe Interactions. Vast microbial omics datasets are needed (1) for metabolic modeling to resolve the interactions that occur within complex, natural microbial communities and (2) for AI models to learn the connections in plant–microbe interactions. Functional data—including strain-level data and data on microbial

Standardized and replicable methods that are widely used within the DOE community would enable researchers to replicate and build on each other's work. The resulting data can easily be integrated for AI analysis.

consortia interactions—linked to plant and soil process data are needed to understand how microbes confer benefits to plants and soil health.

Simulations of Future Crop Performance. Laboratory-scale testing paired with field trials can elucidate plant and cropping system performance under present-day environmental conditions, but integrating current conditions with future projections for temperature, precipitation, and extreme weather is key to identifying the facets of resilience that will be most impactful going forward. This requires agroecosystem models capable of simulating crop performance for predicted environmental conditions, including standard practices for the selection of future scenarios used to run the models. Downscaled projections are necessary to avoid "drizzle bias"—when multiple models' results are averaged to produce ensemble results that lack realistic short-timescale fluctuations in temperature and rainfall— and integrate the likelihood of extreme weather events that future crops must withstand (Gautam et al. 2023).

Soil Data. Field data on feedstock crop effects on soil health parameters across soil types are needed to validate Earth system models and support robust LCA and TEA used to evaluate crop performance. Data on soil organic matter accumulation and persistence are needed, especially as they relate to long-term effects of cultivating perennial feedstock crops.

Data Standards. Community standards for collecting, processing, and documenting data are needed to develop large, shared datasets ready for use in AI/ML models. Standardization will increase the biological signals that models are designed to identify relative to the methodological noise contained in datasets. For example, no consensus has been reached on what constitutes a rhizosphere soil sample, which is expected to support the strongest plant–microbe interactions. Establishing common terminology, definitions, and best practices is key but may never fully address data interoperability and standardization challenges. Developing computational tools and workflows to identify gaps and inconsistencies and harmonize datasets is critical to their use for further modeling and analysis. For example, standardized omics data and bioinformatics tools are needed. Minimum data standards will facilitate combining datasets from different research groups.

Software standards for AI/ML methods are also needed to document methodological improvements and to enable long-term use of the software (e.g., after the student who developed the method graduates). Collaborative efforts among the Bioenergy Research Centers are a useful example of generating large, reproducible, high-quality datasets (bioenergy.org) for training AI/ML algorithms. Learning from these efforts to establish data exchange could enable researchers to balance top-down standards implementation and post-processing approaches for data cleaning and harmonization.

Organizing Existing Data. Collaborations among subject matter experts, data scientists, and computer scientists are needed to overcome challenges in leveraging existing large-scale databases for model inputs. These challenges include variation in spatial and temporal scales across datasets and different discipline-specific terminology referring to the same variables. In addition to USDA soil databases, NASA remote-sensing databases, and National Oceanic and Atmospheric Administration climate databases, centralizing other datasets (e.g., field phenotyping and omics) in databases would accelerate AI/ML efforts.

5.7 Workforce Training Opportunities and Needs

Research progress toward resilient feedstock crop production will require a workforce with multidisciplinary training and experience using state-of-the-art

Key Resources Needed To Accelerate and Enable Crop Resilience Research

- Shared plant resources foundational to engineering plant resilience, such as public genetic and genomic resources, pangenomes and reference databases, transformation facilities, and repositories and biobanks.
- Advances in techniques focused on active microbial taxa, new informatics approaches to resolve functional traits from sequence data, and functional imaging of plant–microbe interactions to identify and manage key beneficial microbial traits.
- Long-term, temporally resolved, multilocation field trials spanning a broad range of environmental conditions to extend and validate controlled-environment research and increase spatial and temporal inference space.
- High-throughput, complementary sensing modalities for plants and microbes that, together with comprehensive environmental monitoring, will facilitate modeling and predictive plant breeding and engineering strategies.
- Standardized data acquisition and processing pipelines for omics and sensing technologies across scales to enable data synthesis across studies and data integration into current and emerging AI/ML applications.
- Innovations in AI/ML approaches to (1) leverage datasets generated by advanced omics technologies and high-throughput phenotyping platforms; (2) reduce data burdens to untangle the effects of genetic, environmental, and management (G x E x M) interactions on plant, microbial, and soil processes; and (3) create computationally efficient surrogates for large-scale models.
- Cross-disciplinary training to prepare the bioeconomy workforce, including providing training in applied AI/ML for feedstock crop research.

technologies and facilities. Three high-priority areas for workforce training were identified.

Cross-Disciplinary Training. The knowledge advances and technological innovations needed to develop resilient feedstock crop production lay at the intersection of traditional disciplinary silos. For example, plant and ecosystem researchers use different disciplinary jargon for phenotyping, hindering the collaboration necessary to understand interactions among plants, soil processes, and environmental conditions. Researchers with skills spanning molecular biology, physiology, agronomy, and data science can bridge these differences to accelerate holistic understanding of feedstock crops.

Applied AI/ML for Resilient Crop Research. Researchers are needed who can bridge communication between experts in applied mathematics and computer science and traditional subject matter experts in plant science, agronomy, soil science, and microbiology. These researchers can build extensive knowledge and skillsets to leverage and adapt off-the-shelf tools while also achieving the depth needed to identify where novel, fit-for-purpose methods should be developed and how best to pursue these efforts. Dedicated resources for workforce development in applied AI/ML will help build a pipeline of researchers with subject matter expertise in resilient feedstock crop research and communication skills to facilitate effective collaboration with AI/ML innovators.

Standardized and Accessible Training Efforts. Shared training programs, including online video

documentation of protocols, will help ensure broad community access to new resources beyond the research groups that developed them. This will also ensure the development and sharing of high-quality collaborative datasets needed to develop AI/ML models.

Training should go beyond using the resources (e.g., plant transformation facilities, high-throughput phenotyping platforms, off-the-shelf AI/ML algorithms, and software packages used for drone imagery and satellite data) to cover data analysis and interpretation.

Appendix A

Workshop Agenda

All times Eastern

Day 1: Wednesday, October 30, 2024

11:00 a.m.	**Welcome and Overview of BER Workshop Series** ***Speakers:*** Dorothy Koch, Associate Director, U.S. Department of Energy Biological and Environmental Research (BER) program; and Todd Anderson, Director, BER Biological Systems Science Division
11:10 a.m.	**Overview of Workshop Goals** ***Speaker:*** Wendy H. Yang (Workshop Co-Chair), University of Illinois Urbana-Champaign
11:15 a.m.	**Plenary I: Biomass Productivity on Likely U.S. Cropland** ***Speaker:*** Emily Heaton, University of Illinois Urbana-Champaign
12:00 p.m.	**Break**
12:15 p.m.	**Overview of Responses to Pre-Workshop Questions** ***Speaker:*** Felix B. Fritschi (Workshop Co-Chair), University of Missouri
12:30 p.m.	**Introduction to Breakout Sessions** ***Speaker:*** Wendy H. Yang
12:40 p.m.	**Breakout 1: Environmental Challenges to Resilient Crop Production** **Goal:** Set the backdrop for the workshop and the report, defining resilient crop production for feedstocks for the bioeconomy. Describe what currently most limits crop productivity and resilience across environmental gradients and present new challenges and research opportunities for the production of nonfood crops cultivated for biomass and seed oil feedstocks.
1:55 p.m.	**Break**
2:25 p.m.	**Consensus Session on Definition of Resilient Crop Production** ***Speaker:*** Felix B. Fritschi

2:40 p.m. **Breakout 1: Key Knowledge Gaps To Achieve Resilient Crop Production for the Bioeconomy**

Goal: Describe the highest priority knowledge gaps that must be addressed to overcome current and anticipated environmental challenges to resilient crop production for the bioeconomy.

3:55 p.m. **Closing Remarks**

Speaker: Wendy H. Yang

Day 2: Thursday, October 31, 2024

11:00 a.m. **Welcome and Highlights from Day 1**

Speaker: Felix B. Fritschi

11:15 a.m. **Plenary 2: Crops and Cropping Systems Resilience: How Can We Effectively Test and Validate Improvements that Are Meaningful?**

Speakers: John Sedbrook, Illinois State University

12:00 p.m. **Break**

12:15 p.m. **Breakout 3: Research Strategies for Tackling Environmental Challenges to Crop Production**

Goal: Propose research strategies to gain fundamental understandings of how to overcome the environmental challenges limiting stable crop production and prioritize the strategies based on how quickly understandings could be gained along with the relative impacts they would have.

1:30 p.m. **Break**

2:00 p.m. **Consensus Session on Diagram of Speed Versus Impact of Proposed Ideas**

Speaker: Wendy H. Yang

2:25 p.m. **Breakout 4: Research Opportunities To Fill Key Knowledge Gaps To Advance Crop Resilience**

Goal: Describe the research required to develop and implement the highest priority strategies identified in Breakout 3 based on how significantly each would impact production for the U.S. bioeconomy.

3:40 p.m. **Closing Remarks**

Speaker: Felix B. Fritschi

Day 3: Friday, November 1, 2024

11:00 a.m.	**Welcome and Highlights from Day 2** ***Speaker:*** Wendy H. Yang
11:15 a.m.	**Plenary 3: Shared Community Resource Development To Support the Implementation of a Resilient Crop System** ***Speaker:*** Jeremy Schmutz, HudsonAlpha Institute for Biotechnology
12:00 p.m.	**Break**
12:15 p.m.	**Breakout 5: Enabling Technologies and Practices Needed To Enable Breakthroughs in Resilient Crop Production** Goal: Describe what innovations are needed in the short to long term to enable breakthroughs in crop resilience research.
1:15 p.m.	**Break**
1:30 p.m.	**Breakout 6: Shared Tools and Resources Needed To Accelerate Development of Resilient Crop Production** **Goal:** Describe the shared resources necessary to enable or accelerate the development of resilient crop production, and how these resources will support research progress.
2:30 p.m.	**Next Steps and Closing Remarks** ***Speaker:*** Felix B. Fritschi

Appendix B

Workshop Participants

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Appendix C

Breakout Questions

Breakout 1

Environmental Challenges to Resilient Crop Production

1. For this report, how do we define resilient crop production?
2. How are environmental challenges, including extreme events, affecting crop production in the short term (i.e., in a given growing season) versus long term (i.e., long-term or legacy effects on yield in the years following the occurrence of a stress event) in your growing region?
3. How are environmental challenges, including extreme events, altering land opportunities for nonfood crops grown for seed oil and biomass feedstocks in your growing region?

Breakout 2

Key Knowledge Gaps To Achieve Resilient Crop Production for the Bioeconomy

Questions for plant-focused groups:

1. What are the key knowledge gaps about genetic diversity, regional zones of adaptation, and identification of key traits that will be needed to develop improved varieties of crops resilient to the stress assigned to your group?
2. What are the most important plant developmental, growth, and physiological processes and responses that we need to better understand in order to enhance crop resilience to the stress assigned to your group?
3. What are the key knowledge gaps about molecular and cellular mechanisms to better understand stress response and the plant resilience trait(s) that would have the highest impact on crop performance? What are the limitations in our understanding of how molecular mechanisms scale to tissue, organ, and whole-plant levels?
4. What are the knowledge gaps underlying plant–microbe interactions that we need to understand to better leverage beneficial microbes to improve stress tolerance and enhance crop resilience?

Questions for microbiome- and ecosystem-focused groups:

1. What are the knowledge gaps underlying plant–microbe interactions that we need to understand to better leverage beneficial microbes to improve stress tolerance and enhance crop resilience?
2. What is most critical for us to learn about beneficial microbes or microbial consortia that confer resilience to the stress assigned to your group that would have the greatest impact on our ability to utilize plant–microbe interactions to increase crop resilience?
3. What research gaps present the greatest obstacles to scaling up crop or cropping system improvements to supply sufficient quantities of feedstock to support an economically resilient U.S. bioeconomy?
4. What uncertainties and data gaps in models (e.g., ecosystem, TEA/LCA, economic) have the greatest impact on our ability to evaluate and set targets for crop production? What gaps in modeling capabilities and input data could be filled by leveraging field studies and other resources?

Breakout 3

Research Strategies for Tackling Environmental Challenges to Crop Production

1. What crop improvements could be made to increase resilience to the environmental challenges described in Breakout 1?
2. How could the microbiome be manipulated to increase crop resilience?
3. What agronomic practices, changes in cropping systems, or new nonfood crops grown as feedstocks or seed oils could help increase the resilience of crop production?
4. Considering the U.S. bioeconomy as a whole, how can we develop a feedstock supply that is more resilient to stresses on crop productivity?
5. How do you rate all the proposed ideas based on how quickly each could be worked out and implemented versus how much impact it would have on increasing crop production for the U.S. bioeconomy?

Breakout 4

Research Opportunities To Fill Key Knowledge Gaps To Advance Crop Resilience

1. To develop and implement the highest priority ideas identified in Breakout 3, what research is most needed?

Breakout 5

Enabling Technologies and Practices Needed To Enable Breakthroughs in Resilient Crop Production

Questions for plant-focused groups:

1. From the crop perspective, what new phenotyping technologies or practices would enable research breakthroughs or significantly impact the pace of discovery?
2. Keeping in mind that there was already a DOE workshop focused on plant transformation and there will be a plant biodesign workshop in 2025, from a practical standpoint, what innovations (e.g., omics, AI/ML, biosensors, robotics) would enable faster development and adoption of more resilient crops?
3. What technologies would enable testing and validation of the effects of plant–microbe interactions on conferring crop resilience to abiotic or biotic stresses?
4. *(Time permitting)* What other technologies or practices are needed to overcome longstanding knowledge gaps about crop resilience and how to improve it?
5. *(Time permitting)* Please categorize the responses in this session as a priority to develop as soon as possible, as an intermediate priority, and as a long-term aspirational goal.

Questions for microbiome- and ecosystem-focused groups:

1. From the microbial perspective, what new technologies or practices would enable research breakthroughs or significantly impact the pace of discovery?
2. From the ecosystem perspective, what new phenotyping technologies, practices, or controlled systems would enable breakthroughs in resilient crop production or significantly impact the pace at which discoveries are made?
3. What other new technologies or practices are needed to overcome longstanding knowledge gaps about crop resilience and how to improve it?
4. What innovations are needed to enable scaling up understanding of resilient crop production from individual plants to field-scale production to predict yield and resilience outcomes for the U.S. bioeconomy?
5. *(Time permitting)* Please categorize the responses in this session as a priority to develop as soon as possible, as an intermediate priority, and as a long-term aspirational goal.

Breakout 6

Shared Tools and Resources Needed To Accelerate Development of Resilient Crop Production

Questions for plant-focused groups:

1. What shared omic resources are needed to enable or accelerate future research and development?
2. What shared crop genetic, germplasm, and microbial resources are needed to enable or accelerate future research and development?
3. What shared infrastructure and tools are needed to test plants and microbiomes under different abiotic stresses across spatial and temporal scales?
4. What resources are needed to improve upon the current state of data processing and data sharing related to plant genetics, omics, and traits, and how will they enable or accelerate future research and development?

Questions for microbiome- and ecosystem-focused groups:

1. What shared crop germplasm and microbial resources are needed to enable or accelerate future research and development?
2. What shared infrastructure and tools are needed to test plants and microbiomes under different abiotic stresses across spatial and temporal scales?
3. What tools or resources are needed to investigate how stresses and crop resilience affect ecosystem processes and their implications for sustainability outcomes?
4. What resources are needed to improve upon the current state of data processing and data sharing related to microbial omics, ecosystem processes, and TEA/LCA, and how will they enable or accelerate future research and development? How could shared resources and coordinated research efforts help reduce uncertainty in models (e.g., ecosystem, TEA/LCA, economic) used to predict broader-scale impacts and to evaluate and set targets for increased resilience in feedstock production?

Appendix D

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Appendix E

Acronyms and Abbreviations

AI	artificial intelligence
AMF	arbuscular mycorrhizal fungi
API	application programming interface
APPL	Advanced Plant Phenotyping Laboratory
ATAC-seq	assay for transposase-accessible chromatin with sequencing
BER	Biological and Environmental Research program
BSSD	Biological Systems Science Division
C	carbon
CABBI	Center for Advanced Bioenergy and Bioproducts
CAM	Crassulacean acid metabolism
CO_2	carbon dioxide
CRISPR	clustered regularly interspaced short palindromic repeats
ddPCR	digital droplet polymerase chain reaction
DOE	U.S. Department of Energy
DRAM	Distilled and Refined Annotation of Metabolism
FAIR	findable, accessible, interoperable and reusable
FAPROTAX	Functional Annotation of Prokaryotic Taxa
Fun-BioCROP	Fixation and Uptake Nitrogen-Bioenergy Carbon, Rhizosphere, Organisms, and Protection
G x E x M	genotype x environment x management
LCA	life cycle assessment
lidar	light detection and ranging
MEMCN	Microbial-Explicit Model incorporating Comprehensive Nitrogen processes
ML	machine learning
N	nitrogen
NASA	National Aeronautics and Space Administration
NIR	near infrared
NUE	nitrogen-use efficiency
PGPR	plant growth–promoting rhizobacteria
qSIP	quantitative stable isotope probing
RGB	red-green-blue
RNA	ribonucleic acid
RNA-seq	RNA sequencing

SWIR	short-wave infrared
SynComs	synthetically assembled communities
TEA	techno-economic analysis
USDA	U.S. Department of Agriculture
VNIR	visible-near infrared
WUE	water-use efficiency
$^{13}CO_2$	carbon-13-labeled carbon dioxide