Perspective

Plant Biosystems Design for a Carbon-Neutral Bioeconomy

Udaya C. Kalluri ¹, **Xiaohan Yang** ¹, and **Stan D. Wullschleger** ²

¹Biosciences Division, Oak Ridge National Laboratory, PO Box 2008, Oak Ridge, TN 37831-6422, USA

²Environmental Sciences Division, Oak Ridge National Laboratory, PO Box 2008, Oak Ridge, TN 37831-6422, USA

Correspondence should be addressed to Stan D. Wullschleger; wullschlegsd@ornl.gov

Received 27 March 2020; Accepted 18 April 2020; Published 11 June 2020

Copyright © 2020 Udaya C. Kalluri et al. Exclusive Licensee Nanjing Agricultural University. Distributed under a Creative Commons Attribution License (CC BY 4.0).

Our society faces multiple daunting challenges including finding sustainable solutions towards climate change mitigation; efficient production of food, biofuels, and biomaterials; maximizing land-use efficiency; and enabling a sustainable bioeconomy. Plants can provide environmentally and economically sustainable solutions to these challenges due to their inherent capabilities for photosynthetic capture of atmospheric CO₂, allocation of carbon to various organs and partitioning into various chemical forms, including contributions to total soil carbon. In order to enhance crop productivity and optimize chemistry simultaneously in the above- and belowground plant tissues, transformative biosystems design strategies are needed. Concerted research efforts will be required for accelerating the development of plant cultivars, genotypes, or varieties that are cooptimized in the contexts of biomass-derived fuels and/or materials aboveground and enhanced carbon sequestration belowground. Here, we briefly discuss significant knowledge gaps in our process understanding and the potential of synthetic biology in enabling advancements along the fundamental to applied research arc. Ultimately, a convergence of perspectives from academic, industrial, government, and consumer sectors will be needed to realize the potential merits of plant biosystems design for a carbon neutral bioeconomy.

Synthetic biology today is poised to tackle a variety of societal challenges. Among these are the daunting needs of addressing climate change mitigation; promoting a biology-based industry that supports production of renewable fuels, chemicals and materials; and launching a sustainable bioeconomy [1–6]. Plant systems studies under the carbon (C) capture context have primarily focused on maximizing the capacity and efficiency of photosynthesis and to a lesser extent on C allocation belowground [2]. Under the bioeconomy context, plant bioengineering efforts have mainly focused on production of biofuels and to a lesser extent on production of fibers, pharmaceuticals, and commodity precursors [5, 6]. Moreover, these limited biological C capture and bioeconomy efforts have relied on distinct deployment pathways. Developing crops dedicated to a single end use, bioenergy or biomaterials or soil sequestration, creates a challenge in competition for the limited land resource. Transformative solutions are needed in order to maximize land use efficiency and accelerate development of environmentally and economically sustainable crops that are efficient in both

sequestration of C and production of economically valuable products. Here, we briefly elaborate on gaps in our process understanding and potential of synthetic biology in enabling a carbon-neutral bioeconomy, and for a defined set of objectives, identify the science and technology needs.

Plants play a central role in biogeochemical cycling of C, with photosynthetic capture of atmospheric CO₂ in leaves, allocation of sugars to above and belowground organs, and partitioning to various chemical forms (primary and secondary metabolites and polymers), including contributions to total soil carbon. There has been a notable increase in the past decade in volume and resolution of information on biological and genetic underpinnings of root chemistry, depth, surface area, fine root production [7], root exudate and root-microbe interactions, root-shoot allometry [8], and plasticity of these traits, at finer resolutions of individual plant and root types. Root chemistry and architecture determine the efficiency of nutrient and water uptake from soil [9], root exudates, interactions with soil microbes, and formation and stability of micro- and macroaggregates [10, 11]. The optimized combinations of root

FIGURE 1: Graphical representation of the plant biosystems design for carbon-neutral bioeconomy concept. Biodesign strategies for maximizing the potential of a carbon neutral bioeconomy are needed to expand the knowledge base along the fundamental to applied research arc and to cooptimize plant chemistry and productivity. Identification of “control knobs” in plant systems, accelerated by advanced synthetic biology approaches and higher throughput in performance efficacy testing (at molecular, plant, and field levels), will enable optimization of genotypes and cultivars that are suited for both above- and belowground contributions.

chemistry and architecture can increase soil organic matter and residence time of C, resulting in an enhancement of C sequestration capability [12, 13]. It is clear that breakthroughs in technological and trait scaling approaches are affording a finer resolution into the mechanisms of C capture in plants and its transformations in soil. Greater investment in clarifying the interconnections among C transformation processes at meso- and microscale to organism and field scales, and underlying genetic controls will be critical in bridging the gaps in process understanding and improving the accuracy of biodesign and modeling efforts (Figure 1). Identification of “control knobs” in plant systems, accelerated by advanced synthetic biology approaches, will enable optimization of genotypes and cultivars that are optimally suited for both above- and belowground contributions. Recently, numerous plant genes associated with root system architecture [14] and root growth rate [15] were identified using genome-wide association studies. More recently, a root-specific gene (*PdNF-YB21*) was shown to increase both root growth and lignin content in poplar [16], which is an important bioenergy crop [17]. These genes could be coordinately expressed, under the control of root-specific promoters [18, 19] to simultaneously increase root biomass, root depth, and root lignin content of crop plants.

Biobased materials and energy derived from renewable, locally-sourced plant biomass are attractive green alternatives to petroleum-derived plastics and composites. Optimal biomass productivity under suboptimal growth conditions is a commonly desirable plant trait to produce feedstocks for biofuels, bioproducts, and biomaterials [20]. The desirable plant traits for distinct end uses are specific, for example, higher cellulose crystallinity and degree of polymerization for nanocellulose-based composites [21, 22], long-chain

unbranched lignin and monomers for plastic precursors [23, 24], and a high aspect ratio and hydrophobicity of fibers for biocomposites [25]. Biomass-based green alternatives can be economically and environmentally sustainable with densification of plant traits conducive for high bioproduct yield as well as productivity on marginal lands. In order to unlock the potential of aboveground plant biomass for generating novel functionality and optimize processing and production of nanocellulose-, lignin- and biofiber-derived materials, it will be critical to expand the knowledge base along the fundamental plant sciences to applied biomaterials research arc [26, 27] (Figure 1). By leveraging the knowledge base of genetic and molecular underpinnings of stem biomass optimization knobs, plant biodesign approaches can potentially enhance product processing outcomes (e.g., accessibility, extractability, unraveling, dispersion, wetting and interfacial binding) for bio-derived monomers, polymers, fibers, and composites and also importantly render biocomponents amenable to novel functionality (acoustic and aesthetic properties) [28–31]. As applications of systems and synthetic biology approaches are showing promise in the fields of bio-derived fuels [32] and synthetic biologic materials such as adhesives and coatings [33], application of synthetic biology approaches is anticipated to facilitate advancements in bio-derived structural materials. For maximizing the potential of a C neutral bioeconomy, biosystems design strategies will need to be developed and implemented to enhance the production of biomass feedstock for bioenergy and biomaterials in the above-ground tissues (e.g., stem, leaf), while simultaneously increasing the storage of C in the below-ground tissues (i.e., roots) (Figure 1).

Plant biosystems design is an emerging interdisciplinary field combining multiple research areas, such as systems

biology, synthetic biology, genetic engineering, plant phenomics, and computational biology. Plant systems design involves iterative cycles of design-build-test-learn. The design step of plant biodesign requires the knowledge of biological components (e.g., composition and function of DNA, RNA, and protein sets) [34] generated by systems biology research as well as the understanding of engineering principles of synthetic biology. The build step will require a community-curated synthetic biology toolbox of theory, parts, and principles to create genetic circuits that can precisely and securely optimize biological control knobs. The test step requires the capability of genetic engineering, systems biology, and plant phenomics for generating multiomics data (genomics, transcriptomics, metabolomics, proteomics, and cellular to field-scale phenomics). The learn step relies on computational biology for integrative analysis of the multiomics data, providing guidance on the improvement of the original biological design.

The design-build-test-learn approach could be used to optimize the engineering of the genes for C capture and the genes for the production of biofuels and bioproducts to achieve appropriate tissue-specific expression. For example, the expression of lignin biosynthesis genes could be down-regulated in the stem tissue to reduce the recalcitrance of biomass to biofuel conversion, while upregulated in the root tissue to increase the lignin content for long-term storage of recalcitrant C in soil. Also, the leaf tissue can be engineered with genes involved in the biosynthesis and accumulation of oils for production of biofuels and lipophilic bioproducts [35–39], genes involved in the biosynthesis of biobased commodity chemicals for production of bioplastics [40], or genes encoding pharmaceutical proteins [41, 42]. On the other hand, simultaneous expression of two genes (*PdGA20ox1* and *PtrMYB221*) driven by a developing xylem tissue-specific promoter increased both quantity and quality (i.e., reduced lignin content) in the aboveground biomass in poplar, without negative impact on root growth [43]. Plant genetic engineering is mainly performed using *Agrobacterium*-mediated transformation, which suffers a limitation in the number of genes that can be transformed each time [44]. As mentioned above, both C capture and biomass feedstock production involve various genes, which could be integrated into the desired locations (e.g., not interfering with the endogenous gene expression) in the plant genome through recombinase-mediated gene integration [45] and tissue-specific gene expression (e.g., leaf-specific gene expression for chemical, stem-specific gene expression for bioenergy feedstock, and root-specific gene expression for C storage). Innovative technological solutions will be needed to develop plant systems and evaluate performance efficacy across cellular to field scales (Figure 1). Towards co-optimizing system performance for simultaneous production of bioenergy, biomaterials, and C storage, an important scientific gap is in our understanding of crosstalk among distal plant organs. Design of synthetic cell-cell and long-distance communication systems [46] can further aid in coordinating the gene expression for optimal spatiotemporal C allocation and partitioning. Facilitation of higher throughput needed in biodesign iterations calls for wider integration

of automation, robotics, artificial intelligence, and predictive modeling-based approaches into the plant science research infrastructure.

Finally, considerations of new technologies including synthetic biology and advanced biodesign should go hand in hand with risk-benefit assessments and ethical, legal, and social implications [47, 48]. Successful integration of the plant-based C biosequestration and plant-derived energy and materials concepts will be influenced by geopolitical and socioeconomic factors. Transforming the pace of progress will need both an increase in consumer demand and an infusion of intentional drivers, such as incentives, mandates, and national targets. Building enabling social ecosystems of people and partnerships across researchers, designers, farmers, industry, consumers, and policy makers will help realize the spectrum of economically and environmentally sustainable solutions [49, 50]. A convergence of perspectives from research, industrial, government, and consumer sectors will be needed to realize the merits of plant biodesign for a C neutral bioeconomy.

Disclosure

This manuscript has been authored by UT-Battelle, LLC under contract no. DE-AC05-00OR22725 with the U.S. Department of Energy. The United States Government retains and the publisher, by accepting the article for publication, acknowledges that the United States Government retains a nonexclusive, paid-up, irrevocable, worldwide license to publish or reproduce the published form of this manuscript, or allow others to do so, for United States Government purposes. The Department of Energy will provide public access to these results of federally sponsored research in accordance with the DOE Public Access Plan (<https://energy.gov/downloads/doe-public-access-plan>).

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Authors' Contributions

UCK, XY, and SDW conceived and wrote the article.

Acknowledgments

This research was sponsored by the U.S. Department of Energy, Office of Science, Biological and Environmental Research, the Center for Bioenergy Innovation and Plant Microbe Interfaces projects. The Laboratory Directed Research and Development Program of Oak Ridge National Laboratory (ORNL) is acknowledged for the opportunity to explore emerging role of synthetic biology in biomanufacturing and develop advanced functional genomics technologies. ORNL is managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract DE-AC05-00OR22725.

References

- [1] L. J. Clarke and R. I. Kitney, "Synthetic biology in the UK – an outline of plans and progress," *Synthetic and Systems Biotechnology*, vol. 1, no. 4, pp. 243–257, 2016.
- [2] C. DeLisi, "The role of synthetic biology in climate change mitigation," *Biology Direct*, vol. 14, no. 1, p. 14, 2019.
- [3] A. A. Myburg, S. G. Hussey, J. P. Wang, N. R. Street, and E. Mizrahi, "Systems and synthetic biology of forest trees: a bioengineering paradigm for woody biomass feedstocks," *Frontiers in Plant Science*, vol. 10, p. 775, 2019.
- [4] M. Naseem, O. Osmanoglu, and T. Dandekar, "Synthetic rewiring of plant CO₂ sequestration galvanizes plant biomass production," *Trends in Biotechnology*, vol. 38, no. 4, pp. 354–359, 2019.
- [5] P. M. Shih, "Towards a sustainable bio-based economy: redirecting primary metabolism to new products with plant synthetic biology," *Plant Science*, vol. 273, pp. 84–91, 2018.
- [6] J. C. Mortimer, "Plant synthetic biology could drive a revolution in biofuels and medicine," *Experimental Biology and Medicine*, vol. 244, no. 4, pp. 323–331, 2019.
- [7] N. E. Robbins 2nd and J. R. Dinnyen, "Growth is required for perception of water availability to pattern root branches in plants," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 115, pp. 822–831, 2018.
- [8] P. P. Handakumbura, B. Stanfill, A. Rivas-Ubach, D. Fortin, J. P. Vogel, and C. Jansson, "Metabotyping as a stopover in genome-to-phenome mapping," *Scientific Reports*, vol. 9, no. 1, article 1858, 2019.
- [9] K. S. Pregitzer and A. L. Friend, "The structure and function of Populus root systems," in *Biology of Populus and its Implications for Management and Conservation*, pp. 331–353, NRC Research Press, Ottawa, 1996.
- [10] E. K. Morris, D. J. P. Morris, S. Vogt et al., "Visualizing the dynamics of soil aggregation as affected by arbuscular mycorrhizal fungi," *The ISME Journal*, vol. 13, no. 7, pp. 1639–1646, 2019.
- [11] K. Zhahnina, K. B. Louie, Z. Hao et al., "Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly," *Nature Microbiology*, vol. 3, no. 4, pp. 470–480, 2018.
- [12] D. A. Martens, "Plant residue biochemistry regulates soil carbon cycling and carbon sequestration," in *Soil Biology and Biochemistry*, vol. 32, no. 3, pp. 361–369, 2000.
- [13] B. Adamczyk, O. M. Sietiö, P. Straková et al., "Plant roots increase both decomposition and stable organic matter formation in boreal forest soil," *Nature Communications*, vol. 10, no. 1, 2019.
- [14] D. Ristova, M. Giovannetti, K. Metesch, and W. Busch, "Natural genetic variation shapes root system responses to phytohormones in *Arabidopsis*," *The Plant Journal*, vol. 96, no. 2, pp. 468–481, 2018.
- [15] N. Bouain, A. Korte, S. B. Satbhai et al., "Systems genomics approaches provide new insights into *Arabidopsis thaliana* root growth regulation under combinatorial mineral nutrient limitation," *PLoS Genetics*, vol. 15, no. 11, p. e1008392, 2019.
- [16] Y. Zhou, Y. Zhang, X. Wang et al., "The root-specific NF-Y family transcription factor, PdNF-YB21, positively regulates root growth and drought resistance by ABA-mediated IAA transport in *Populus*," *The New Phytologist*, 2020.
- [17] X. Yang, U. C. Kalluri, S. P. DiFazio et al., "Poplar genomics: state of the science," *Critical Reviews in Plant Sciences*, vol. 28, no. 5, pp. 285–308, 2009.
- [18] J. S. Jeong, Y. S. Kim, K. H. Baek et al., "Root-specific expression of OsNAC10 improves drought tolerance and grain yield in rice under field drought conditions," *Plant Physiology*, vol. 153, no. 1, pp. 185–197, 2010.
- [19] C. Mohan, A. N. Jayanarayanan, and S. Narayanan, "Construction of a novel synthetic root-specific promoter and its characterization in transgenic tobacco plants," *3 Biotech*, vol. 7, no. 4, p. 234, 2017.
- [20] J. Bailey-Serres, J. E. Parker, E. A. Ainsworth, G. E. D. Oldroyd, and J. I. Schroeder, "Genetic strategies for improving crop yields," *Nature*, vol. 575, no. 7781, pp. 109–118, 2019.
- [21] A. Kafy, H. C. Kim, L. Zhai et al., "Cellulose long fibers fabricated from cellulose nanofibers and its strong and tough characteristics," *Scientific Reports*, vol. 7, no. 1, article 17683, 2017.
- [22] R. J. Moon, A. Martini, J. Nairn, J. Simonsen, and J. Youngblood, "Cellulose nanomaterials review: structure, properties and nanocomposites," *Chemical Society Reviews*, vol. 40, no. 7, pp. 3941–3994, 2011.
- [23] J. Wang, R. Boy, N. A. Nguyen et al., "Controlled assembly of lignocellulosic biomass components and properties of reformed materials," *ACS Sustainable Chemistry & Engineering*, vol. 5, no. 9, pp. 8044–8052, 2017.
- [24] N. A. Rorrer, D. R. Vardon, J. R. Dorgan, E. J. Gjersing, and G. T. Beckham, "Biomass-derived monomers for performance-differentiated fiber reinforced polymer composites," *Green Chemistry*, vol. 19, no. 12, pp. 2812–2825, 2017.
- [25] M. Fortea-Verdejo, E. Bumbaris, C. Burgstaller, A. Bismarck, and K. Y. Lee, "Plant fibre-reinforced polymers: where do we stand in terms of tensile properties?," *International Materials Reviews*, vol. 62, no. 8, pp. 441–464, 2017.
- [26] D. M. Alonso, S. H. Hakim, S. Zhou et al., "Increasing the revenue from lignocellulosic biomass: maximizing feedstock utilization," *Science Advances*, vol. 3, no. 5, article e1603301, 2017.
- [27] A. K. Mohanty, S. Vivekanandhan, J. M. Pin, and M. Misra, "Composites from renewable and sustainable resources: challenges and innovations," *Science*, vol. 362, no. 6414, pp. 536–542, 2018.
- [28] A. Bourmaud, J. Beaugrand, D. U. Shah, V. Placet, and C. Baley, "Towards the design of high-performance plant fibre composites," *Progress in Materials Science*, vol. 97, pp. 347–408, 2018.
- [29] A. Gallos, G. Paes, F. Allais, and J. Beaugrand, "Lignocellulosic fibers: a critical review of the extrusion process for enhancement of the properties of natural fiber composites," *RSC Advances*, vol. 7, no. 55, pp. 34638–34654, 2017.
- [30] M. J. Le Guen, S. Hill, D. Smith et al., "Influence of rice husk and wood biomass properties on the manufacture of filaments for fused deposition modeling," *Frontiers in Chemistry*, vol. 7, p. 735, 2019.
- [31] H. L. Tekinalp, V. Kunc, G. M. Velez-Garcia et al., "Highly oriented carbon fiber-polymer composites via additive manufacturing," *Composites Science and Technology*, vol. 105, pp. 144–150, 2014.
- [32] U. C. Kalluri, H. Yin, X. Yang, and B. H. Davison, "Systems and synthetic biology approaches to alter plant cell walls and

- reduce biomass recalcitrance,” *Plant Biotechnology Journal*, vol. 12, no. 9, pp. 1207–1216, 2014.
- [33] R. A. Le Feuvre and N. S. Scrutton, “A living foundry for synthetic biological materials: a synthetic biology roadmap to new advanced materials,” *Synthetic and Systems Biotechnology*, vol. 3, no. 2, pp. 105–112, 2018.
- [34] X. Yang, L. S. Qi, A. Jaramillo, and Z. M. Cheng, “Biodesign research to advance the principles and applications of biosystems design,” *BioDesign Research*, vol. 4, 2019.
- [35] M. Pyc, Y. Cai, M. S. Greer et al., “Turning over a new leaf in lipid droplet biology,” *Frontiers in Plant Science*, vol. 22, no. 7, pp. 596–609, 2017.
- [36] T. Vanhercke, S. Belide, M. C. Taylor et al., “Up-regulation of lipid biosynthesis increases the oil content in leaves of *Sorghum bicolor*,” *Plant Biotechnology Journal*, vol. 17, no. 1, pp. 220–232, 2019.
- [37] T. Vanhercke, U. K. Divi, A. El Tahchy et al., “Step changes in leaf oil accumulation via iterative metabolic engineering,” *Metabolic Engineering*, vol. 39, pp. 237–246, 2017.
- [38] O. Yurchenko, J. M. Shockey, S. K. Gidda et al., “Engineering the production of conjugated fatty acids in *Arabidopsis thaliana* leaves,” *Plant Biotechnology Journal*, vol. 15, no. 8, pp. 1010–1023, 2017.
- [39] X. R. Zhou, S. Bhandari, B. S. Johnson et al., “Reorganization of acyl flux through the lipid metabolic network in oil-accumulating tobacco leaves,” *Plant Physiology*, vol. 182, no. 2, pp. 739–755, 2020.
- [40] E. Jongedijk, S. Müller, A. D. J. van Dijk et al., “Novel routes towards bioplastics from plants: elucidation of the methylperillate biosynthesis pathway from *Salvia dorisiiana* trichomes,” *Journal of Experimental Botany*, no. article eraa086, 2020.
- [41] L. Kopertekh and J. Schiemann, “Transient production of recombinant pharmaceutical proteins in plants: evolution and perspectives,” *Current Medicinal Chemistry*, vol. 26, no. 3, pp. 365–380, 2019.
- [42] F. Sainsbury, “Innovation in plant-based transient protein expression for infectious disease prevention and preparedness,” *Current Opinion in Biotechnology*, vol. 61, pp. 110–115, 2020.
- [43] J. S. Cho, H. W. Jeon, M. H. Kim et al., “Wood forming tissue-specific bicistronic expression of *PdGA20ox1* and *PtMYB221* improves both the quality and quantity of woody biomass production in a hybrid poplar,” *Plant Biotechnology Journal*, vol. 17, no. 6, pp. 1048–1057, 2019.
- [44] S. B. Gelvin, “Agrobacterium-mediated plant transformation: the biology behind the “gene-jockeying” tool,” *Microbiology and Molecular Biology Reviews*, vol. 67, no. 1, pp. 16–37, 2003.
- [45] V. Srivastava and J. Thomson, “Gene stacking by recombinases,” *Plant Biotechnology Journal*, vol. 14, no. 2, pp. 471–482, 2016.
- [46] J. Andres, T. Blomeier, and M. D. Zurbriggen, “Synthetic switches and regulatory circuits in plants,” *Plant Physiology*, vol. 179, no. 3, pp. 862–884, 2019.
- [47] K. E. French, “Harnessing synthetic biology for sustainable development,” *Nature Sustainability*, vol. 2, no. 4, pp. 250–252, 2019.
- [48] S. B. Jaconis, A. T. Morita, P. Coutinho, and S. Borschiver, “Systematically monitoring, relational database and technology roadmapping for trends and innovation opportunities in biopolymers,” *Journal of Renewable Materials*, vol. 7, no. 11, pp. 1221–1230, 2019.
- [49] L. D. Anadon, G. Chan, A. G. Harley et al., “Making technological innovation work for sustainable development,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 113, no. 35, pp. 9682–9690, 2016.
- [50] N. E. Matthews, C. A. Cizauskas, D. S. Layton, L. Stamford, and P. Shapira, “Collaborating constructively for sustainable biotechnology,” *Scientific Reports*, vol. 9, no. 1, article 19033, 2019.