Bioenergy Underground: Challenges and opportunities for phenotyping roots and the microbiome for sustainable bioenergy crop production

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Abbreviations: BRCs, Bioenergy Research Centers; CP-MAS, cross polarization magic-angle spinning; CT, computed tomography; DBTL, design-build-test-learn; ECM, ectomycorrhizal; EcoFABs, fabricated ecosystem devices; FTIR, Fourier-transform infrared; GC-MS, gas chromatography-mass spectrometry; GHG, greenhouse gas; LC-MS/MS, liquid chromatography-tandem mass spectrometry; NMR, nuclear magnetic resonance; RSA, root system architecture; SOM, soil organic matter; SRL, specific root length.

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**Abstract**

Bioenergy production often focuses on the aboveground feedstock production for conversion to fuel and other materials. However, the belowground component is crucial for soil carbon sequestration, greenhouse gas fluxes, and ecosystem function. Roots maximize feedstock production on marginal lands by acquiring soil resources and mediating soil ecosystem processes through interactions with the microbial community. This belowground world is challenging to observe and quantify; however, there are unprecedented opportunities using current methodologies to bring roots, microbes, and soil into focus. These opportunities allow not only breeding for increased feedstock production but breeding for increased soil health and carbon sequestration as well. A recent workshop hosted by the USDOE Bioenergy Research Centers highlighted these challenges and opportunities while creating a roadmap for increased collaboration and data interoperability through standardization of methodologies and data using F.A.I.R. principles. This article provides a background on the need for belowground research in bioenergy cropping systems, a primer on root system properties of major U.S. bioenergy crops, and an overview of the roles of root chemistry, exudation, and microbial interactions on sustainability. Crucially, we outline how to adopt standardized measures and databases to meet the most pressing methodological needs to accelerate root, soil, and microbial research to meet the pressing societal challenges of the century.

1 INTRODUCTION

As the transition away from fossil fuels and toward a biomass-based fuel economy accelerates, improving the economic viability and ecological sustainability of bioenergy cropping systems is increasingly critical. Annual seed-based biofuel feedstocks such as maize (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.), which have dominated the U.S. bioenergy portfolio to date, are high-yielding and profitable but often fail to provide substantive climate benefits relative to fossil fuels (Hochman & Zilberman, 2018). As climate change mitigation has become a central tenet in the bioenergy initiative, it is imperative that bioenergy cropping systems provide net greenhouse gas (GHG) reduction through avoided emissions and increased soil carbon (C) storage (Robertson et al., 2017). To lessen competition with food agriculture, bioenergy cropping systems should also be targeted toward lands that are marginally productive for traditional agriculture, including regions that are prone to drought, landscape positions that are susceptible to excessive moisture, and systems where soil amendments are uneconomical or impractical (Yang et al., 2021).

Focusing on the root system of bioenergy crops and association with the soil microbiome has potential to accelerate crop productivity and sustainability, especially on marginal lands. Roots serve as the primary interface for water, nutrient, and energy transactions among plants, microbes, and soils. Belowground traits play central roles in mediating soil health, plant nutrient acquisition, feedstock stress resistance, and subsequent aboveground plant yields (Figure 1) (Bitterlich et al., 2018; Boddey et al., 2003; Hartman & Tringe, 2019; Keymer & Kent, 2014; Smith & Read, 1996). The rhizosphere is the region of soil influenced by roots, where a holistic framework is needed to understand the integrated dynamics of roots, exudates, soil, and the microbial community (York et al., 2016). Feedstock trait values optimizing biomass allocation to roots, root system architecture (RSA), root functional dynamics, and symbiotic associations reduce the need for exogenous fertilizer and water inputs (Bakker et al., 2012), thus fostering productivity, resilience, and negative GHG budgets central to the effective deployment of bioenergy systems. However, root and microbiome phenotyping, or measuring, remains a significant impediment to realizing these positive effects that would optimize bioenergy cropping systems for marginal lands (Figure 1).

1.1 Bioenergy crops for climate mitigation

When managed properly, bioenergy cropping systems have the potential to mitigate climate change through their effect on GHG fluxes and C sequestration in soils (Robertson et al.,
Core Ideas

- Root and belowground traits have been overlooked in bioenergy feedstock research.
- Developing core traits and standardized protocols will facilitate bioenergy research.
- Methods exist now to facilitate belowground research for bioenergy sustainability.
- Data fusion across bio-scales needs multivariate analyses for interpretation.
- Collaboration and getting our hands dirty will be needed for the Bioenergy Underground.

1.2 Targeting root traits for bioenergy cropping systems

Despite the obvious importance of root and microbiome traits in determining the sustainable productivity of bioenergy cropping systems, belowground traits remain poorly studied because of the spatiotemporal dynamics of soil–root–biota interactions, micro- or millimeter scale, and sampling disturbance that present methodological challenges, especially in the opaque and difficult to access soil environment. Expansion of these research efforts will require transdisciplinary collaboration to overcome current methodological hurdles.

Root system plasticity, both architecturally and physiologically, is caused by soil properties, interactions with microbiota, and climatic conditions (Hodge, 2009), which makes designing proper experimental controls challenging. Many reductive ex situ approaches solve this challenge, making it possible to study root traits at very fine scales, but come up against numerous methodological inaccuracies that make the translation to field conditions uncertain. Moreover, even though the importance of deep roots has been highlighted in several reviews (Germon et al., 2020; Jackson et al., 1996; Pierret et al., 2016), the functional significance of deep roots is commonly underestimated in situ due to truncated soil sampling depths (Freycon et al., 2015). Overall, the inherently destructive nature of most sampling techniques and variation in the measurement types at all scales of experimental design still represent sizable impediments to the investigation of root phenotypes (Zeng et al., 1998; Kleidon & Heimann, 2000).

Incorporating root phenotyping into bioenergy feedstock development presents an underutilized opportunity to optimize sustainable bioenergy agroecosystems. As the bioenergy movement continues forward, identifying the most economically viable and GHG-negative biofuel feedstocks will require coordinated and streamlined approaches to root phenotyping of physical, chemical, and biological traits. In this manuscript, trait will refer to a general measurable phene (e.g., root angle), while trait value will refer to a particular phene state (e.g., steep) (York et al., 2013). Phenotype refers to the totality of an organism’s observable trait values. The process to utilize optimized belowground phenotypes will entail intensive collaboration between researchers across disciplines to overcome the methodological challenges presented by root phenotyping research. On 5 March 2021, a root phenotyping workshop was convened by the four USDOE Bioenergy Research Centers (BRCs) to initiate sharing of methodologies and discussion of future needs and collaboration regarding belowground research. To further this process, we describe pressing research questions, current challenges, and areas of
Dominant traits that should be optimized belowground in bioenergy crops include those affecting root system architecture, chemistry and exudation, nutrient transport, and the microbiome. These belowground optimizations will lead to CO₂ capture, increased production and income, and improved soil health and carbon sequestration.

**FIGURE 1** Dominant traits that should be optimized belowground in bioenergy crops include those affecting root system architecture, chemistry and exudation, nutrient transport, and the microbiome. These belowground optimizations will lead to CO₂ capture, increased production and income, and improved soil health and carbon sequestration.

Opportunity for advancements in root phenotyping of bioenergy crops, applicable to other systems as well.

### 2 RATIONALE FOR STANDARDIZING METHODS/APPROACHES FOR BIOENERGY ROOT RESEARCH

Root traits are inherently plastic to environmental conditions and differ between annuals and perennials and between herbaceous and woody feedstocks. The lack of a standardized set of sustainability traits, as well as a lack of data integration tools to connect root traits to belowground functional processes, represent challenges to the elucidation of the role of root systems in feedstock sustainability and ecosystem function.

Obtaining a unified suite of belowground measurements for bioenergy crops would aid in addressing these challenges, further providing the foundation to develop meta-analyses of environment–species interactions and provide data streams to inform models (e.g., DayCent, AgroIBIS, FUN-BioCROP) predicting ecosystem function. The National Science Foundation-funded NEON program ([www.neonscience.org](http://www.neonscience.org)) represents an example of a data network designed to monitor the long-term health and function of ecosystems (Teeri & Raven, 2002). Although extensive in approach, the challenges of measuring root traits have...
limited NEON assessment of root characters to root biomass and chemistry sampled on 5-yr interval timescales. Coordination of research activities involving belowground measures, including time- and trait-intensive measures, would provide valuable information not only on bioenergy crop influence on ecosystems, but also on the effects of longer-term environmental change and management activities on feedstock sustainability and bioenergy crop ecosystem function.

2.1 Targeting root phenotypes

Central to developing a research blueprint and set of coordinated measures is the identification of a core set of traits that reliably identify the structural and functional attributes of roots. Variation in root traits exists among genotypes of the same species and across species due to intrinsic genetic differences, and this genetic capacity can be harnessed for feedstock improvement through breeding and genetic modification. Root plasticity describes the capacity of the root system to change form and function in reaction to external conditions (Hodge, 2009). Root plasticity itself may also be under genetic control, and although plasticity may be important for organism acclimation to the environment, researchers need to be careful assuming that any observed plasticity is necessarily beneficial (Schneider & Lynch, 2020). These core traits need to be readily measured and standardized across the research community. In addition, complementarity and integration among root traits and the microbial community are important in evaluating root response and feedstock productivity and function in varying environments (Freschet et al., 2020; York et al., 2013).

Recent advances in imaging, image processing, and micrometeorological sensor technologies have generated an increasing number of highly multidimensional datasets alleviating previous limitations in large-scale phenotypic data acquisition that constrained breeding advances (Araus & Cairns, 2014). Functional root traits can be targeted to reduce energy requirements (Colombi et al., 2019; Guo et al., 2021), improve crop tolerance to environmental stressors (Comas et al., 2013; Lynch, 2019; Guo & York, 2019; Klein et al., 2020), improve yields (Wu et al., 2004), and reduce environmental effects of production, all critical aspects of the sustainability of bioenergy feedstocks.

Direct measures of roots are not the only traits relevant to feedstock development, however. Indirect effects of roots on biogeochemical cycles and other aspects of the surrounding ecosystem are important and in need of standardization as well. Previously, rhizosphere has been proposed as an extended phenotype of the root (York et al., 2016). Even measures as relatively fundamental as respiration of roots and heterotrophs in soil are not reported in standardized formats across studies and systems. Adopting standard methods for both sampling and measurement will allow easier quantitative comparisons between field sites or entirely different biomes (Ryan & Law, 2005; Kim et al., 2019). The standardization of procedures embedded in the acquisition and analytical pipelines of belowground data would facilitate cross-comparisons among studies, improve downstream analysis of data, and accelerate the development of the next generation of multiscale simulation models.

Several existing databases provide guidance for trait selection, including the TRY database (Kattge et al., 2020) and Fine-Root Ecology Database (Iversen et al., 2017). In TRY, aspects of root system function can be gleaned from measures of fine root branching intensity, fine root dry mass per ground area, and fine root length per soil volume (root length density). TRY also contains accessions on root chemical and physiological traits, such as fine root calcium content per fine root dry mass, fine root phenols content per fine root dry mass, and fine root phosphatase activity per fine root dry mass. These databases can serve as a starting point for trait selection and provide guidance for cataloging root traits valuable for sustainability researchers.

2.2 Root traits for selection

The adoption of core root traits and their measurement across environments and genotypes will ultimately provide targets for breeding and genetic modification. While phenotypic targets for feedstock improvement have been identified for aboveground traits such as water-use efficiency and yield (McKown et al., 2014; Chhetri et al., 2019), targets for important belowground traits such as rooting density, exudation, or other traits associated with resilience, productivity, and C sequestration of feedstock ecosystems have not yet been highlighted. The reliance on quantitative trait loci and genome-wide association study approaches require an extensive evaluation of traits across broad feedstock genetic stocks. These approaches, central to the identification of loci and single-nucleotide polymorphisms conferring trait attributes, require numerous phenotypic observations across diverse genotypes and, if seeking environmental plasticity, across varying environmental conditions. This data collection intensity has been an impediment to understanding the role of root traits in feedstock performance. The development of high-throughput phenotypic platforms and advanced analytical capabilities, such as artificial intelligence, will pave the way for the next green revolution of targeted breeding (Atkinson et al., 2019). Functional phenomics offer a relevant research paradigm that uses phenotypic diversity and large multivariate datasets to both address genetic mapping and ecophysiological understanding using multivariate data analysis, detailed physiological studies, and simulation modeling in a pipeline that includes both hypothesis generation and testing (York, 2019).
As it stands, gaining a functional and predictive understanding of belowground root mediated traits that enable the growth of bioenergy crops in marginal lands and improve the sustainability of these crops is lacking. Leveraging collaboration across BRCs and across the entire root research community could provide potential solutions to the challenges in studying these complex belowground traits critical to ecosystem processes. Large-scale replication across a set of differing soil types can allow us to determine the stochastic and consistent root phenotypic responses. Fundamentally, BRCs share primary strategies of sustainability, feedstock development, deconstruction/separation, and conversion. These strategies could be the foundation to build upon a set of standardized experiments, results for comparison, and replication to capture the plasticity that roots display in an altered environment.

3 | ROOT SYSTEM TRAITS AND FUNCTION

The efficacy of root systems in resource acquisition to support biofeedstock production and their capacity for C sequestration in soils will be reflected in a suite of traits from the whole plant, to the cellular, to the biochemical levels. These traits, root system architecture, root anatomy, physiology, and rhizosheath development, may be modified by plant response to environmental conditions and to the soil microbial community and may vary broadly by species and among genotypes within feedstock species.

3.1 | RSA

Root system architecture refers to the three-dimensional spatial deployment of roots over time (Lynch, 1995) and is influenced by plant developmental processes and genetics but also by environmental stimuli. Root plasticity is frequently observed as proliferation in localized patches of nutrients or due to soil physical constraints (Schneider & Lynch, 2020). Developmentally, RSA is largely determined by the dominant root classes, as defined by the taxonomy of the International Society for Root Research (Zobel & Waisel, 2010). All plants growing from a seed have a tap root that emerges first, and which can produce lateral roots that can further branch to have more laterals. Although this structure dominates in dicot plants, the grasses also form shoot-borne roots that can emerge from leaf nodes. Grass RSA is further complicated by tillering and rhizomes that lead to highly complicated root systems, especially in perennial grasses. Because all plants from seeds grow tap roots, and many types of roots are small diameter and fiber-like, we recommend avoiding ambiguous terms like tap-rooted or fibrous to describe root systems.

The RSAs of perennial woody feedstock species differ from those of herbaceous plants in the development of large-diameter, lignified roots formed for structural support and resource storage. These perennial structures are the site of fine root production—roots involved in resource acquisition—and the vagaries of measuring root topology and geometry have limited the understanding of the function of woody-plant root systems (Pregitzer et al., 2002; Danjon & Reubens, 2008). Effective foraging space has been defined as the soil volume in which 80% of root activity occurs and may be an aggregate measure useful for woody species (Wahid, 2000). Since effective foraging space is functional in nature, it is a valuable concept when considering feedstock production on marginal soils or for soil C sequestration by feedstocks.

Regardless of species, the lateral roots are the sites of many of the main activities considered in feedstock production: metabolic activity, water and nutrient acquisition, mycorrhizal colonization and microbiome community activity, and rhizodeposition (Pregitzer et al., 2002; Cumming et al., 2015), thus making their assessment of central importance in bioenergy feedstock research.

3.2 | Root morphology, anatomy, and physiology

Root anatomical traits, the cross-sectional structure of the root tissues, may play important roles in feedstock productivity, especially on marginal soils. Overall root diameter, root hair density and length, and the characteristics of the xylem influence water and nutrient uptake, the development of rhizosheaths, and connectivity between soil and root (Bengough et al., 2016; Haling et al., 2016; Carminati et al., 2017; Ruiz et al., 2020). Root hairs, for example, increase the effective surface area of the root, and variation in root hair density and length may represent adaptive traits that will contribute to resource acquisition (Carminati et al., 2017; Ruiz et al., 2020). Root hairs are important in soil water extraction as they increase soil contact, increase the effective root diameter, and reduce the diffusive limits to soil water flux (Carminati et al., 2017), although the significance of root hairs cannot be generalized (Cai et al., 2021) and should be evaluated for feedstock species/genotypes of interest. The role of root hairs in nutrient acquisition and soil nutrient extraction is better defined. The ability of root hairs to bridge root–soil surfaces, penetrate small diameter soil pores, and increase the effective root diameter increases the uptake of nutrients, especially those of low diffusivity, such as phosphate (Bates & Lynch, 2000; Haling et al., 2013; Ma et al., 2001).
Variation in xylem number, diameter, and length influence root hydraulic conductance, and extensive variation occurs between and within species as well as with a plant and, in the case of woody species, during the root’s lifespan (McElrone et al., 2004; Kirfel et al., 2017). Such differences in xylem anatomy represent functional adaptations to water availability (Tyree et al., 1994) and may represent significant targets for selection for feedstock production under water limitation. Many cross-sectional anatomical traits related to the area and activity of the cortex are related to the concept of metabolic burden, by which C use of roots can be reduced (Galindo-Castañeda et al., 2018).

Another, more direct measure of metabolic burden is root respiration as CO$_2$ release. Recently, a high-throughput method was developed for measuring specific root respiration and used for the first genetic analysis of this potentially valuable trait (Guo et al., 2021). Another physiological trait of direct relevance for roots and sustainability is nutrient uptake, which can be measured as the rate of depletion of a nutrient from solution (Griffiths & York, 2020). A new medium-throughput assay for the uptake of multiple nutrients indicated that substantial heritable variation existed within maize that can be harnessed for direct breeding or genetic modification (Griffiths, Roy, et al., 2021). Physiological traits are a frontier of untapped potential for use of root traits for bioenergy sustainability.

3.3 | Rhizosheaths

Rhizosheaths consist of soil particles held together by root hairs, mucilage, and other exudates produced by the root and associated rhizosphere microbial community (Watt et al., 1994). Rhizosheaths represent a bridge between the root surface and surrounding soil and play roles in water and nutrient acquisition by providing connectivity of flow paths from the soil. Often produced by cereals and grasses in response to soil stress, they are also a component of other species’ root systems as well and vary among genotypes.

There are many factors involved in rhizosheath formation, including root hair length and density (Burak et al., 2021), root and microbial activity (Barre & Hallett, 2009), and soil moisture and texture (Haling et al., 2014; Liu, Ye, et al., 2019). Comparison of five switchgrass ecotypes exposed to drought stress showed a greater rhizosheath weight of ‘Alamo’ compared with ‘Kanlow’ and other ecotypes, and this was correlated with the presence of denser root and longer root hairs in Alamo (Liu, Chen, et al., 2019).

3.4 | Root systems of bioenergy crops

As root characteristics are central traits that support resource acquisition, vary extensively within and between species, and are influenced by the environment and microbial symbionts, here we highlight some of the important root system features of several bioenergy crops being researched by BRCs.

3.5 | Sorghum root system

Sorghum [Sorghum bicolor (L.) Moench] is a perennial C4 grass that is grown annually for grain production, animal forage, and bioenergy feedstock production. Energy sorghum, a photoperiod sensitive cultivar, grows more than 3 m tall with a maximal rooting depth surpassing 3 m, while producing 10–20 Mg ha$^{-1}$ of aboveground biomass on marginal soils, and 7 Mg ha$^{-1}$ of belowground biomass (Fu et al., 2016; Tang et al., 2018; Schetter et al., 2021).

The tap root emerges from the seed to form the primary root system, which remains dominant for the first few weeks postemergence. Sorghum does not produce secondary seminal roots from the embryo, as closely related relatives do (such as maize). The secondary root system consists of shoot-borne roots that emerge from leaf nodes over time and that can proliferate large numbers of lateral roots. Nodal roots from the first five to seven nonelongating internodes are described as crown roots. Nodal roots from higher nodes that reach the ground are termed brace roots, and roots from even higher nodes that do not reach the ground are termed aerial roots. All nodal roots produce a heterogenous viscoelastic polysaccharide mucilage that can be colonized by N-fixing bacteria.

3.6 | Switchgrass root system

In contrast to annual energy sorghum, switchgrass (Panicum virgatum L.) is a perennial bioenergy feedstock species. Switchgrass grown from seed starts with a single shoot and tap root system, but that forms shoot-borne roots, rhizomes, and rhizome-borne shoots and roots over time, leading to a dense root system of fine roots. However, the shoot-borne roots of switchgrass are noticeably thicker compared with other grasses. In the upland cultivars ‘Sunburst’ and ‘Dacotah’ grown over 3 yr in North Dakota, the root system to a soil depth of 110 cm accounted for nearly 27% of the total plant biomass and up to 84% with the crown (Frank et al., 2004). In perennial grasses, the crown represents the basal area of the plant where the stems and roots are connected. New tillers and roots start from the crown.

Differences in root characteristics were reported among switchgrass types with the upland types having a higher root density than the lowland types (Ma et al., 2000). Comparison of root architecture among different switchgrass cultivars showed a variation in the range of 150% in the specific root length (SRL; length per gram dry weight), with the lowland types Alamo and Kanlow having coarser roots compared with
the upland cultivars ‘Forestburg’, ‘Carthage’, and Sunburst (Ma et al., 2000; Graaff et al., 2013). The root hair length and density of Alamo were found to be greater than those of Kanlow (Liu, Chen, et al., 2019). Profiles of root distribution in plot studies across eight locations showed that root mass averaged approximately two-thirds of the annual harvest of aboveground biomass and that approximately 50 and 75% of switchgrass roots in the top 90 cm of soil can be found in the top 15 and 30 cm of the soil profile, respectively (McLaughlin et al., 1999). Deeper rooting in switchgrass was found to be associated with greater N uptake using stable isotopes across two ecotypes grown in both low N and water tall mesocosms (Griffiths, Wang, et al., 2021).

3.7 | Poplar root system

Considering its central role as a woody biofuels feedstock, it is surprising that so little work has focused on root system architecture in the genus *Populus*. One challenge, of course, is researching such a large and woody root system. Another issue stems from the two propagation approaches that may influence studies of root architecture: seedling vs. cutting. While evaluation of seedling root system architecture and root response to environmental factors is similar to approaches taken with *Arabidopsis* (e.g., Shinde et al., 2017, 2019), feedstock propagation is typically via cuttings, which will be the focus here.

Root mass and extent are all strongly correlated with the size of the cutting used to establish the tree (Douglas et al., 2016). In addition, extensive clonal variation in root number, length, and mass suggest lines could be selected for feedstock production on marginal soils (Phillips et al., 2014; Douglas et al., 2016; Carthy et al., 2018). This variation has additionally been used to help identify genes associated with rooting propensity (e.g., Dash et al., 2017, 2018), establishing pathways for selection of root traits.

3.8 | The response of root system architecture to abiotic stresses

Plant roots exposed to limiting soil moisture, nutrients, and other soil factors respond by modulating root traits, such as branching and root length, to maximize the acquisition of resources (Quinn et al., 2015; Yu et al., 2019). A study of maize recombinant inbred lines comparing plants with contrasting lateral root number and length grown under water stress showed that the lines with fewer but longer roots had 51–67% greater shoot biomass at flowering and 144% greater yield than lines with many short roots (Zhan et al., 2015). Although these results reflect intrinsic genotypic differences, they suggest plastic responses that increase root branching density may improve drought tolerance. Plasticity in the branching ability and depth of the root system has also been described in rice (*Oryza sativa* L.) in response to drought. As with maize, rice genotypes having the ability to develop deep roots were more drought tolerant than those lacking deep roots (Kameoka et al., 2015; Menge et al., 2016).

Root architecture in *Populus* is also environment dependent. In drought studies, root development was dependent on soil saturation (Carthy et al., 2018), and lateral root growth was inhibited by PEG exposure, which may be controlled by the *PtaZIP1*-like (Dash et al., 2017) and *PtaJAZ3* and *PtaRAP2.6* (Dash et al., 2017, 2018) genes. Root biomass density, length, surface area, and SRL were also stimulated by N fertilization (Yan et al., 2019), and SRL and fine:coarse root ratio were strongly influenced by the form of N used (Domenicano et al., 2011; Yan et al., 2019), reflecting the plasticity of root architectural traits in *Populus*. Understanding the genes and networks underlying root architectural change to the environment will allow for future selection for stress resistance.

Rhizosheaths, often correlated with root hair length, increase soil-root contact and increase effective root diameter, and variation in rhizosheath development may be utilized to identify the genetic underpinnings of root characteristics that could be used to increase feedstock water- and nutrient-acquisition efficiency (Delhaize et al., 2015; James et al., 2016; Lynch et al., 2014). Rhizosheath formation in switchgrass is stimulated by water limitation, with the Alamo ecotype exhibiting the greatest production, and this increase in rhizosheath formation was accompanied by significant increases in root hair length and density and root sugar and amino acid concentrations (Liu, Ye, et al., 2019). Clearly, root rhizosheath formation represents a potentially fruitful target for feedstock development.

3.9 | Phenotyping root system architecture

Phenotyping methods for RSA can be divided into destructive and nondestructive methods and have been exhaustively reviewed (Paez-Garcia et al., 2015; Topp et al., 2016). Destructive methods are the standard for root measurements to allow direct access to entire roots. These methods usually require at least destroying a portion of the root system, such as with root ingrowth cores (Andreasson et al., 2016) and soil coring (Burridge et al., 2020) but can also involve the destructive harvest of the entire root system, such as from pot studies (Griffiths, Wang, et al., 2021). In all cases, roots are washed free from debris and scanned on flatbed scanners that produce high contrast images for further analyses described below. Root crown phenotyping (or “shovelomics,”; Trachsel et al., 2011) is another destructive method during which the top portion of root systems (root crowns) are extracted using a shovel, cleaned of debris, and the root crown is imaged for further
These types of destructive methods are by far the most prevalent in the literature, but the nondestructive methods remain a critical need for time-series studies of growth.

Minirhizotrons and rhizoboxes are used to observe and image roots. They both rely on roots that are embedded in soil and grow along transparent surfaces. Minirhizotrons are transparent acrylic tubes that are installed at an angle into soil in the field such that specialized cameras may be inserted to acquire images along the face of the tube. In this way, root dynamics can be observed in the field, including root birth and death. For greenhouse studies, rhizoboxes are rectangular structures with usually a thin layer of soil with a clear acrylic window and are placed at an angle such that roots may grow along the surface of the window for observation. In the past, manual tracing and tracking was used (Möller et al., 2019), but recent advances in image analysis may allow striking progress in their widespread adoption (Smith et al., 2020; Wang et al., 2019).

Rhizotron and rhizobox methods rely on roots growing along artificial surfaces for observation, which are known to have an influence on root growth. Therefore, a fascinating frontier of root research is the use of various electromagnetic signals that can penetrate soil and nondestructively reveal roots, such as nuclear magnetic resonance (NMR), X-ray computed tomography (CT), and radar. Using NMR, the water in and around plant roots is mapped to create two- or three-dimensional images of the root systems. High-field NMR can image root systems in high detail, but structured soil provides background interference (van Dusschoten et al., 2016). Low-field NMR can image root systems in similar detail, but it is less affected by paramagnetic elements in the soil, thus allowing for imaging of root systems in a wide range of natural soil types (Bagnall et al., 2020). This technique provides a nondestructive way to observe the root system of plants, however it is currently time-intensive, requires the use of soil cores or a rhizotron system, and has a minimal resolution of ~1 mm, meaning fine roots may be difficult to resolve. X-ray CT uses X-rays from multiple angles to compile 3D volumes of root systems embedded in soil (Tracy et al., 2010). Currently, microCT systems can generate images with pixels as small as 100 nm, relevant for fine roots; however, the nature of X-ray attenuation means that roots may be difficult to segment from the soil because of similar intensity. Nuclear magnetic resonance and X-ray represent the frontier for detailed reconstructions of 3D root systems over time, but these approaches are presently constrained by being mostly limited to laboratory use and imaging columns or pots.

For field use, ground penetrating radar is the most studied nondestructive method for roots, during which electromagnetic pulses are broadcast into the ground (Guo et al., 2013). The pulses scatter back to the instrument receiver when they cross boundaries of contrasting materials, where the received information can be used for 3D reconstruction. This nondestructive technique can be applied to large areas of a field for high-throughput phenotyping. However, this technique is unable to reconstruct finer root details currently, so it is best used for larger belowground structures such as in tuber crops, trees, and nodal roots. Another promising approach for radar may be not attempting 3D reconstruction, but rather using the signals directly for estimating root length density, which may also predict length of finer roots in aggregate (Liu et al., 2018). Various electrical methods have also been proposed but generally require sensors to be placed in the ground or on the plant (Peruzzo et al., 2020).

### 3.10 Root system architecture image analysis

Roots are imaged across a wide degree of modalities, such as in situ with minirhizotrons or soil pits, in rhizoboxes, on colored backgrounds, or with flatbed scanners (Topp et al., 2016). Therefore, image analysis approaches are often specific to particular types of collected images. Software that works with 2D branched and connected root system images such as root crowns or seedling root systems grown on blue paper included EZ-Rhizo (Armengaud, 2009), SmartRoot (Lobet et al., 2011), RootNav (Pound et al., 2013), and ARIA (Pace et al., 2014). In many cases, roots are not connected as they have been excavated from the field or pots, and these roots are typically imaged on a flatbed scanner then analyzed using the commercial WinRhizo software (Regent Instrument Inc.). However, the previous de facto standard for root measurements, WinRhizo, was shown to drastically underestimate root volume when root diameters within an image vary because the software uses a volume formula using total root length and the average root diameter (Rose, 2017). Most recently, RhizoVision Explorer was released as a ready-to-run executable for Windows that facilitates interactive root image analysis as well as batch analysis (Seethepalli et al., 2021). RhizoVision Explorer correctly calculates root volume, and analyzes both connected root systems, such as seedlings, as well as disconnected roots washed from soil cores or pots. However, RhizoVision Explorer and WinRhizo require high contrast root images for successful root identification. RootPainter (Smith et al., 2020) uses machine learning after initial training to segment roots from more complex backgrounds, such as in minirhizotrons or rhizoboxes, and these segmented root images can then be used with RhizoVision Explorer. The combination of RootPainter, or similar tools, for segmenting complex imagery with RhizoVision Explorer for feature extraction is a promising pipeline for future root research.

The frontiers of RSA phenotyping include broadening the base of scientists who are root-enabled by releasing
open-source software tools, sharing standardized protocols, and pushing the frontiers of nondestructive methodologies. Knowledge gaps include how phenotyping roots among plant ages or environments affects results, or whether phenotyping vegetatively propagated plants is indicative of seed-grown plants. Because root systems represent a substantial carbon expenditure for the plant, carbon is potentially diverted from the root system to achieve higher shoot biomass yields during selection. However, it is unknown if selection on aboveground biomass in bioenergy crops tends to decrease the relative allocation to the root system.

4 | ROOT STRUCTURAL CHEMISTRY, METABOLITES, AND EXUDATES

In addition to root traits, root biochemical characteristics and rhizosphere modifications represent critical traits that affect both biofeedstock productivity and ecosystem function (York et al., 2016). These root traits vary greatly across and within species and life forms and are modified by environmental conditions (Silver & Miya, 2001).

4.1 | Root chemistry influences soil C dynamics

Root chemistry, including broad traits such as C:N ratios and specific traits such as carbohydrates (i.e., cellulose and hemicellulose) profile and lignin structures and content, affect decomposition rates and therefore, potentially contribute to SOM stocks. For example, C:N and lignin are negatively correlated with root decomposition rates, whereas holocellulose content is positively correlated with root decomposition for perennial bioenergy grasses (Austin et al., 2009; von Haden et al., 2019; Xu et al., 2020). The compositions of roots and its chemical features also vary depending on the root resources. Although these correlations are not universal (Austin et al., 2009), the potential of root chemistry to influence SOM is important because total root biomass can exceed shoot biomass in some species (Poorter et al., 2012). Indeed, root-derived SOM may exceed that derived from shoots by 2.4 times (Rasse et al., 2005; Kong & Six, 2010). Further, microorganisms can process and incorporate root C into biomass four times faster than aboveground plant residues (Rasse et al., 2005). Given current thinking that microbially derived necromass, metabolites, and decomposition products, rather than recalcitrant plant material, account for the majority of stabilized SOM (Simpson et al., 2007; Grandy & Neff, 2008; Kleber & Johnson, 2010; Schmidt et al., 2011), the faster cycling of root material through microbial biomass can lead to greater SOM accrual. Thus, root chemistry plays a significant role in influencing the process rates and decomposer community structure and, ultimately, the accrual of C by biofeedstock systems.

4.2 | Root metabolites

Fundamental differences in the metabolism of bioenergy feedstock species and genotypes may reflect fundamentally different ecological capacities and/or responses to environmental factors. Plant acclimation to stress often includes specific sets of metabolic adjustments, including changes in primary and secondary metabolites as well as the establishment of reactive oxygen species-scavenging responses (Faroog et al., 2009; Michaletti et al., 2018). While such metabolic fingerprints may be used as a trait for bioenergy feedstock selection, especially for use on marginal soils, data on plant metabolic profiles is largely limited to leaves (Bogeat-Triboult et al., 2007; Hamanishi et al., 2010, 2015; Tschaplnski et al., 2019).

Primary and secondary metabolites and levels of antioxidant compounds are often reflective of metabolic activity and stress response. Switchgrass lines differing in rhizosheath production and drought response exhibited root amino acid, sugar, and organic acid profiles that significantly changed in response to water limitation, with arginine, isoleucine, methionine, and cysteine and kestose, raffinose, fructose, fucose, sorbose, and xylose increasing as rhizosheath size increased (Liu, Chen, et al., 2019). Metabolic profiles in roots of switchgrass also change in response to nutrient stress (Ding et al., 2021). In Populus nigra L., water limitation altered C allocation to the roots, with concomitant changes in C partitioning between nonstructural carbohydrate pools and induction of antioxidant enzymes (Regier et al., 2009). In Populus deltoides, water stress differentially affected the flavonoids chrysirin, myricetine, kaempferol, and isofulvic acid in roots of different genotypes and may reflect the adaptability of poplar plants to water stress (Popovic et al., 2016).

In addition to influencing feedstock environmental stress resistance, such broad differences in root metabolic profiles may influence rhizodeposition (below) as well as the functional ecology of the rhizosphere (Clemensen et al., 2020). Thus, metabolic assessment and engineering may represent avenues to enhance feedstock sustainability and control agroecosystem GHG fluxes.

4.3 | Root exudates

As another important root trait, rhizodeposits include passively released low molecular weight compounds, active exudation of secondary metabolites, proteins, and mucilage, and sloughed root cap and border cells (Uren, 2007). Rhizodeposition accounts for 10–40% of primary productivity (Nguyen, 2003), but of the components comprising rhizodeposits, root
exudates have received the most attention. Root exudates are organic compounds including both primary (e.g., sugars, amino acids, organic acids, fatty acids) and secondary metabolites (including volatile organic compounds and plant hormones), that shape a variety of plant and soil traits ranging from root growth and architecture to soil physical and chemical properties (e.g., soil pH, nutrient content, water holding capacity) (Bais et al., 2006; Baudoin et al., 2003; Canarini et al., 2019; Sasse et al., 2018; Shi et al., 2011). Dynamic interactions between roots and soil microorganisms mediated by root exudates can benefit plant growth and stimulate plant water and nutrient acquisition while enhancing the resistance of plants to biotic and abiotic stressors, creating emergent properties and traits (Rolfe et al., 2019). Therefore, root exudates and the rhizosphere should be considered part of the root extended phenotype (Canarini et al., 2019; York et al., 2016).

4.4 Root exudates respond to the environment

Rhizodeposition is both responsive to and alters the environment (Canarini et al., 2018; Dakora & Phillips, 2002; de Vries et al., 2019). Exudation is altered by drought, with broad changes in profiles depending on plant species, where increases may reflect active responses of plants to modify the rhizosphere or increased deposition as roots lose cell integrity and die (Preece & Peñuelas, 2016). Other studies have documented exudation shifts to sugars, antioxidants, and secondary compounds under drought (Gargallo-Garriga et al., 2018). Changes in plant nutrient status can also lead to changes in the exudation rates of various carbohydrates, organic acids, and amino acids relative to each other and in total (Carvalhais et al., 2011; Desai et al., 2014; Higa et al., 2010; Kräffczyk et al., 1984; Naik et al., 2009; Vranova et al., 2013). These changes in rhizodeposition may allow bioenergy feedstock species to engineer their soil environments, build rhizospheraths, or otherwise modify the rhizosphere to aid in the acquisition of water and limiting nutrients, with concomitant effects on ecosystem processes including C storage and nutrient cycling.

4.5 Root exudates affect the soil microbiome and vice versa

In addition to altering rhizosphere water and nutrient relations, chemical composition of root exudates can act as a selective agent on the rhizosphere microbiome, altering microbial community structure and soil ecosystem function (Gschwendtner et al., 2011; Shi et al., 2011). Through exudates, plants can communicate with and regulate microbial communities, stimulating beneficial microbes and inhibiting the growth of pathogenic microbes (Bais et al., 2006; Sasse et al., 2018; Shinde et al., 2017; Vranova et al., 2013). At the same time, the composition of the soil microbial community also influences rhizodeposition (Korenblum et al., 2020) through changes in gene expression induced in the root by symbionts/associations (Labbé et al., 2011; Shinde et al., 2019). Indeed, different root microbiomes often exhibit fundamentally different rhizodeposition profiles (Klugh & Cumming, 2007; Klugh-Stewart & Cumming, 2009; Desai et al., 2014), which may influence feedstock productivity and sustainability on marginal soils.

In the rhizosphere, root exudates represent an easily accessible source of labile organic C. This readily available C influences microbial population size, activity, and community structure (Eilers et al., 2010; Gschwendtner et al., 2011; Haichar et al., 2008; Shi et al., 2011) and may also accelerate the mineralization of SOM in an effect referred to as priming (Kuzyakov et al., 2000). Much of the priming effect observed in the rhizosphere is likely the result of microbes mining SOM for N while using plant C for metabolism and biomass construction (Bradford et al., 2008; Cheng & Coleman, 1990; Cheng & Kuzyakov, 2015; Nobili et al., 2001). On the face of it, this priming effect would seem to have a detrimental effect on SOM stocks, but this is usually not the case. Large quantities of root exudates may select for microbial communities dominated by fast growing, copiotrophic species (Boddy et al., 2007; Fierer et al., 2007). The resulting faster microbial biomass turnover of copiotrophs would increase the quantity of microbial residues generated and thus available for the formation and accrual of SOM (Cotrufo et al., 2019; Wieder et al., 2014). In addition, root exudates themselves can be rapidly incorporated into SOM as the exuded low-molecular weight compounds easily sorb to mineral surfaces (Bradford et al., 2008).

As we consider root chemistry as part of the multidimensional root phenotype, there remain significant gaps in our knowledge, leaving pressing research questions. Although there is already evidence of links between root chemistry and the rhizosphere microbiome, given the importance of this microbiome for plant health and productivity our knowledge is still in its infancy, especially with respect to bioenergy cropping systems. There remain many questions surrounding mechanisms by which root exudates shape microbial community assembly and function. For example, some biological nitrification inhibition compounds are released via secondary transport (Di et al., 2018; Egenolf et al., 2021). However, it is generally not known how these transport mechanisms mediate the response of root exudation to rhizosphere soil conditions. We have also observed that low soil moisture can potentially induce changes in root exudate quantity and composition due to plant stress response and can affect the movement of hydrophilic vs. hydrophobic root-derived compounds to alter the effect of root exudates on the microbial community and feedstock species to engineer their soil environments, build rhizospheraths, or otherwise modify the rhizosphere to aid in the acquisition of water and limiting nutrients, with concomitant effects on ecosystem processes including C storage and nutrient cycling.

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The analysis of lignin, cellulose, and hemicellulose content is most often determined using wet-analytical methods, but several studies have highlighted improvements in experimental throughput by NIR techniques (Elle et al., 2019). Lignin structural features are commonly reported by $^{13}$C quantitative NMR or heteronuclear single quantum coherence of isolated samples, although solid-state $^{13}$C cross polarization magic-angle spinning (CP/MAS) has also been frequently employed (Clemente et al., 2013; Rencoret et al., 2020). The latter approach does frequently suffer from spectral overlap, whereas heteronuclear single quantum coherence minimizes this problematic issue. Raman imaging has been used to map-out the location of lignin in the root cell wall. Fourier-transform infrared (FTIR) spectroscopy has also been used as a companion method, but the structural information is limited in comparison to NMR methodologies. A hydrolysis-ion chromatography protocol is most often used to determine carbohydrate sugar profiles in roots. New data generated broadly by the use of these methods will provide insights into how root tissue chemistry is altered as bioenergy crops are engineered for biorefining of aboveground biomass, with implications for soil C and rhizosphere microbial communities.

Given the importance of root exudates, it is critical that we use a wide-range of analytical methods to assess variation in rhizodeposition among feedstocks and the potential roles of exudation in environmental response. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) and gas chromatography-mass spectrometry (GC-MS) are leading approaches due to their ability to characterize complex mixtures with relatively high sensitivity. However, these approaches typically require liquid or solid phase extraction to fractionate metabolites from salts and other sources of interference and metabolite identifications are typically based on comparison with authentic standards. NMR, on the other hand, can directly determine the structures of novel compounds without this reliance on standards, however, it is less suitable for complex mixtures and low abundance compounds.

While LC-MS/MS, GC-MS, and NMR are used for analyses of ex situ samples, there are a range of spectroscopic methods, especially FTIR (Legner et al., 2018; Li et al., 2021) that can enable direct in situ analysis of chemical groups, albeit by looking at bulk spectroscopic features vs. the structures of constituent molecules. Fourier-transform infrared can also provide information on the localization of chemical classes within the rhizosphere which can be complemented by using mass spectrometry imaging to localize metabolites (Sasse et al., 2020). Stable isotopes provide a complementary method to determine the temporal rates of production of exudates, for example, by introducing $^{13}$CO$_2$ into a gas tight growth chamber. The resulting isotopically labeled metabolites can be resolved from the $^{12}$C metabolites to determine when particular metabolites are produced and even calculate exudation rates. In addition, the $^{13}$C-labelled roots will greatly enhance the NMR signals thus enabling the characterization of low abundance components or metabolites.

We see several opportunities to standardize analytical methods for the analysis of exudates across institutions, especially LC-MS/MS and GC-MS analyses. First, the use of common growth conditions including experimental systems such as fabricated ecosystem devices (EcoFABs) (Sasse et al., 2019) that facilitate exudate collection to help ensure comparable samples are generated. Similarly, standardization of exudate extraction and analytical methods can allow community researchers to directly compare results and ideally leverage authentic standard libraries for compound identification. Finally, by comparing results across diverse plant species we can define components of core exudomes for bioenergy crops to define internal standards that can be used for metabolite identification and quantification. Specifically, spiking in isotopically labeled versions of core exudome metabolites can significantly improve the ability to confidently identify and quantitatively profile these metabolites. Internal standards are especially important in complex sample matrices such as exudates and rhizosphere samples that contain interferences that may suppress the ionization of metabolites of interest. Overall, by adopting common protocols for exudate collection and analysis, including internal standards, research will be accelerated by enabling scientists to compare data and build on each other’s results.

The rhizosphere represents the critical interface between plants, microbes, and soil (Bakker et al., 2013). Plants mediate and drive belowground biological interactions and activities in the rhizosphere through root exudation (Inderjit et al., 2003). These exudates, along with other rhizodeposits, stimulate microbial activity and help drive synergistic activities of diverse and functional groups of soil microbes, including N-fixing bacteria, P-solubilizing siderophore-producing microbes, as well as arbuscular mycorrhizal fungi and...
ectomycorrhizal (ECM) fungi, which help promote plant growth and resilience through provisioning plant nutrition and by priming plant immunity (Li et al., 2020; Martin-Rivilla et al., 2020; Wang & Feng, 2021; Jiang et al., 2021).

The root microbiome includes rhizospheric bacteria and fungi that are less intimately associated with roots, rhizoplane organisms that may colonize and form biofilms along all plant root surfaces, and symbiotic partners that all affect plant health and growth (Asanuma et al., 1979; Balasundararajan & Dananjeyan, 2019; Kumar & Singh, 2020). Rhizoplane microbiota may survive by exchanging metabolites with the plant; however, most are not specialized to invade plant cells or apoplastic tissues (Sattelmacher, 2001). However, endophytic microbiota are able to evade plant defenses and live within plant tissue as symbionts (Provorov, 2009; Dini-Andreote, 2020). These include the well-known nodulating N-fixing bacteria, arbuscular mycorrhizal fungi and ECM fungi, as well as a diversity of other bacteria and fungi (Berthelot et al., 2019; Doty et al., 2009; Ghimire et al., 2009; Wang et al., 2016). Rhizoplane and endophytic members of the root microbiome participate in direct signaling and molecular communication with the host plant and may aid in damping stress signals, including reactive oxygen species, provision of macro- or micronutrients, or protecting the host through chemical means (Torres et al., 2012; Babu et al., 2014). Such intimate plant-microbe signaling can involve diverse molecules, including low molecular weight compounds, small secreted peptides, and lipochitooligosaccharides, altering plant development and response to the environment (Plett et al., 2014; Rush et al., 2020). Rhizoplane and endophytic microorganisms are also active in acquiring and transporting nutrients and water out of soil for their plants’ hosts, thus, they may directly and indirectly improve stress-tolerance of plants growing in marginal environments. In this way, their activities can help to contribute to ecosystem-scale sustainability benefits of bioenergy crops such as improving soil structure and C stability and reducing soil N and C emissions (Kell, 2011; Moore et al., 2021). Thus, belowground root-microbe biology, communication, and dynamics are fundamental to plant health and the development of sustainable bioenergy cropping systems.

Much of our understanding about root biology comes from reductive experimental systems or greenhouse experiments, which have limited translation to field scale ecosystems. This limitation is in part due to difficulties capturing spatial and temporal variation in root systems and complicated by limited understanding of how the wide range of traits relevant to belowground processes influence each other (Freschet et al., 2020). For example, both root chemistry and architecture are known to influence microbial community composition, while common microbial mutualists, such as mycorrhizal fungi, have in turn been shown to influence root chemistry and architecture, yet there is little work correlating root traits to microbiome structure and function (Cruz et al., 2004; Bais et al., 2006; Pervaiz et al., 2020; Saleem et al., 2018).

Design, build, test, learn (DBTL) is an ideal framework for both top-down and bottom-up microbiome design in closed systems due to the regulation of environmental conditions (Lawson et al., 2019). However, the success of the engineered single-species microbes within complex ecological networks, such as the rhizosphere, is likely limited due to the extensive functional redundancy, environmental variability, and emergent properties characteristic of the rhizosphere microbiome. Moreover, many endophytic and rhizosphere microorganisms have not been cultured, leaving gaps in functional profiles of plant-associated microbiota (Compant et al., 2010).

An alternative approach is to engineer microbial recruitment and recognition mechanisms into bioenergy germplasm. For example, transgenic Arabidopsis thaliana expressing a Populus trichocarpa lectin-receptor-like kinase protein (PiLecRKL1) involved in the ECM symbioses, displayed ECM fungal sheaths despite A. thaliana being a non-ECM species (Labbé et al., 2019). Lectin receptor-like kinase proteins are pattern recognition receptors that bind to microbial-associated molecular patterns and initiate Populus symbiosis with ECM fungi, Laccaria bicolor (Sun et al., 2020). These results suggest pattern recognition receptors may be a target for engineering plant symbioses (Cope et al., 2021). Unfortunately, the molecular mechanisms governing plant perception of microbial symbionts, specifically within bioenergy species, require extensive investigations. DBTL can be used to methodically dissect the genetic and regulatory elements, as well as signaling pathways involved in the specific recruitment of plant-growth-promoting rhizobacteria and fungi. Identification of the interspecies signaling involved in the recruitment of target bacterial and fungal taxa provides an opportunity to incorporate sustainable root-microbial traits into bioenergy germplasm development.

Some of these questions are being pursued amongst the BRCs, including recruitment and turnover of rhizosphere microbiomes, how their distribution and activity change with depth, and what taxa constitute core (shared) communities. In these investigations, central themes become apparent, such as seeking strategies to visualize and localize specific microbial taxa, the importance of biofilms, and utilizing mixed fungal/bacterial communities. All of these aspects are limited by currently available methods and variance in what measurements are considered relevant across the discipline.

5.1 Engineering plants for bioenergy: effects on the root microbiome

Within bioenergy science, there is a significant focus on engineering of bioenergy crops with reduced feedstock recalcitrance, which typically involves changes in cell wall
composition, to facilitate conversion to fuel. Several studies have shown that changes in cell wall composition alter plant-microbe interactions and, likewise, plant-microbe interactions cause changes in plant cell wall composition (Beckers et al., 2016; Gutjahr et al., 2015; Salas-González et al., 2021). In addition, several groups are engineering bioenergy crops that accumulate bioproducts or bioproduct precursors (e.g., for renewable and biodegradable plastics) (Lin & Eudes, 2020; Lin et al., 2021). Lignin is an important component in these interactions and is also the target of many bioenergy crop engineering projects. However, very little is known about how the engineering for improved downstream processing affects plant resilience to the environment and interactions with microbes.

Although many projects are aimed at reducing recalcitrance, such as by reducing lignin in above-ground biomass, there is also an increasing interest in minimizing GHG emissions and increasing soil C sequestration of bioenergy crops (e.g., by increasing compounds such as lignin or suberin in roots). However, accumulation of such plant-derived compounds in the roots or soil must be studied in the context of soil processes and the rhizosphere microbiome to understand how the changes in root properties affect microbiome composition and the production of relatively stable soil C (Bailey et al., 2019; Dynarski et al., 2020).

Bioenergy researchers are interested in whether we can engineer plants or their microbial partners to create a more adaptive root microbiome to benefit plant performance in marginal environments and soils. However, there is currently insufficient knowledge to rationally design plants for recruitment of specific microbiomes. We do anticipate, through the DBTL framework, that it will be possible to engineer plants to produce specific exudates that stimulate certain microorganisms over others to promote plant growth and soil C sequestration, while reducing GHG emissions and increasing the value of bioenergy crops.

6 SYNTHESIS

The primary focus of bioenergy feedstock improvement efforts over the past two decades has been on aboveground chemistry and productivity. The goals of sustainable bioenergy crop production and enabling a C-neutral bioeconomy will, however, require consideration of both above- and belowground plant system performance (Weigelt et al., 2021). Plant root systems and the surrounding soil environments that they influence, or rhizospheres, are complex, dynamic ecosystems that affect bioenergy crop yields and soil health (York et al., 2016). While root traits have long been considered a phenotypic target in plant breeding (O’Toole & Bland, 1987), the complexities of belowground ecosystems and the difficulties accessing them have hindered our understanding of these key environments. With dozens of reports on new root imaging technologies and image analysis software emerging from the research community in the past decade, we are collectively better poised now than ever to represent the “hidden half” in agroecosystem studies that consider the aboveground and belowground simultaneously (Atkinson et al., 2019) but have to separate the hype from the useful. While researchers often report that roots are the “hidden half” and difficult to study, they should understand that roots can successfully be studied for contributions to scientific knowledge using readily available methods, even while we wait for the promises from frontier technologies.

There remains a dearth in approaches that have both streamlined higher-throughput data acquisition and analysis of RSA, especially under field conditions (Brunner et al., 2019; Downie et al., 2015; Hodge, 2004; Trachsel et al., 2011; Tracy et al., 2020). Root phenotyping technologies offer tradeoffs from lower throughput, high-resolution to higher throughput and lower resolution, and there is a growing demand in the community for versatile and accessible technologies that are in the sweet spot of higher throughput and resolution. The broader research community has a significant role to play in driving the expectations and standards for empirical methodology, metadata and data quality and storage such that bioenergy crop data generated from across species, field sites, growth matrices, and phenotyping methodologies are conducive for robust meta-studies and modeling efforts.

Advancements in plant and root system characterization technologies are, however, creating opportunities for integrated experimentation across the scientific community that can expedite progress in multi-scale understanding on how root structural and chemical traits are influenced by plant genetics, climatic conditions, and edaphic factors. One such advancement is in the development of fabricated ecosystems that are designed to link observations across benchtop, greenhouse, and field experiments. These systems range in size, cost and complexity, from a small hand-held size, laboratory EcoFABs to those that can encompass entire facilities (e.g., EcoTrons, EcoPODs) (Lawton et al., 1993; Singer et al., 2021; Dowd et al., 2021). These engineered ecosystems could be especially useful for studying the increased below and aboveground production found in mixed assemblages of grassland species (Yang & Tilman, 2020). Democratized technologies and access to new integrated lab and field-scale facilities as a community resource will allow for the integration of root system architecture, microbiome composition, chemical mass balance, soil matrix, and exudate measurement or sampling technologies into existing research pipelines while increasing the throughput of testing responses to abiotic (environmental) and biotic (plant and microbe) changes, thus accelerating hypothesis generation and testing (Sasse et al., 2019; Zengler et al., 2019).
Recent proliferation of phenotyping and measurement technologies is resulting in a dramatic increase in the number, heterogeneity, and complexity of datasets. Understanding features, spatiotemporal patterns, and correlations from large heterogeneous datasets requires use of appropriate statistical and data analysis methods. The community needs to embrace multivariate methods (Paliy & Shankar, 2016), because too often multivariate data is treated with many univariate analyses. Machine learning methods are often advocated due to their ability to find nonlinear connections in data that can offer substantial predictive value, however these methods can often be a black box that lack interpretability so crucial for increasing scientific understanding. Therefore, more exploration of interpretable machine learning (Azodi et al., 2020), or explainable artificial intelligence, will be needed for the success of these proposed research programs across bio-scales.

Concerted community efforts are needed to be able to bridge bio-scales of experimental studies and maximize utility of plant phenotypic data collected towards modeling efforts. To effectively accelerate our understanding of how root architecture, rhizosphere function, soil health, and plant yields are linked to and influence one another, standardized data collection and reporting is needed. This is particularly true when trying to integrate research conducted in laboratory ecosystems with field observations collected from minirhizotrons or imaging of root architecture of excavated plants (Gray et al., 2013; York & Lynch, 2015). Considerations should be made when collecting data on the types of data (e.g., RSA), the scale of measurements, and the methods of measurement. This is particularly important to facilitate effective simulation modelling of these systems. Selection of the core traits and creation of a protocol library will be essential to the success of this program. It is encouraging to already see an organic increase in community synergy around root imaging approaches, standardizing nomenclature, and usage of root image data sharing platforms (Lobet et al., 2013). The plant science community at this juncture needs similar synergy in the context of root chemistry characterization. Adoption of standard protocols for root and exudate collection and chemical analysis as well a commitment to F.A.I.R. data principles (Mons et al., 2017) in analytical chemistry data and library sharing for root, rhizosphere, and soil analyses will catalyze meaningful meta-studies and integration of independently generated datasets into models. In a broader context of root phenotyping for fundamental plant or applied food, fuel, or fiber crops research, scientific societies and funding agencies can be powerful catalysts in promoting and incentivizing coordination of research endeavors and leveraging alignment within and across funding agencies.

7 | CONCLUSION

Ultimately, we envision a belowground bioenergy research community collecting data across sites and experiments to build community knowledge even when pursuing individual aims (Figure 2). Many core root traits can be collected now in almost any lab using destructive but simple soil coring, washing, scanning, dry weighing, and analysis with
RhizoVision Explorer. These traits include root length density, specific root length, average diameter, and root tissue density, which have recently been described in a guide on the methods and theory of root trait ecology (Freschet et al., 2021). In the longer term, nondestructive root phenotyping tools should continue development until they are ready for wider community adoption. Measuring core rhizosphere traits, also referred to as the rhizosphere metaphenome (Jansson & Hofmockel, 2018), which includes chemical, soil, and microbiome characteristics such as metabolomes, proteomes, transcriptomes, and metagenomes, as well as microbial functions and responses to the environment, will require coordinated research hubs and partnerships among bioenergy research centers. These core trait measurements will require standardized protocols and data sharing platforms. The core message of the Bioenergy Underground is that we need to get our hands dirty and get digging to realize the unprecedented opportunities to increase feedstock production on marginal lands while improving carbon sequestration to combat the climate emergency (Intergovernmental Panel on Climate Change, 2021).

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CONFLICTS OF INTEREST
The authors declare no conflicts of interest.

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REFERENCES

Andreasons, F., Gonzalez, M., Augusto, L., & Bakker, M. R. (2016). Comparison of ingrowth cores and ingrowth meshes in root studies: 3 years of data on Pinus pinaster and its understory. Trees, 30(2), 555–570.

Araus, J. L., & Cairns, J. E. (2014). Field high-throughput phenotyping: The new crop breeding frontier. Trends in Plant Science, 19(1), 52–61.

Armengaud, P. (2009). EZ-Rhizo software: The gateway to root architecture analysis. Plant Signaling & Behavior, 4(2), 139–141.

Asanuma, S., Tanaka, H., & Yatazawa, M. (1979). Rhizoplane microorganisms of rice seedlings as examined by scanning electron microscopy. Soil Science and Plant Nutrition, 25(4), 539–551. https://doi.org/10.1080/0380768.1979.10433195

Atkinson, J. A., Pound, M. P., Bennett, M. J., & Wells, D. M. (2019). Uncovering the hidden half of plants using new advances in root phenotyping. Current Opinion in Biotechnology, 55, 1–8.

Austin, E. E., Castro, H. F., Sides, K. E., Schadt, C. W., & Classen, A. T. (2009). Assessment of 10 years of CO2 fumigation on soil microbial communities and function in a sweetgum plantation. Soil Biology and Biochemistry, 41(3), 514–520. https://doi.org/10.1016/j.soilbio.2008.12.010

Azodi, C. B., Tang, J., & Shiu, S.-H. (2020). Opening the black box: Interpretable machine learning for geneticists. Trends in Genetics, 36(6), 442–455.

Babu, A. G., Shim, J., Bang, K.-S., Shea, P. J., & Oh, B.-T. (2014). Tri- choderma virens PDR-28: A heavy metal-tolerant and plant growth-promoting fungus for remediation and bioenergy crop production on mine tailing soil. Journal of Environmental Management, 132, 129–134.

Bagnall, G. C., Koonjoo, N., Altobelli, S. A., Conradi, M. S., Fukushima, E., Kueethe, D. O., Mullet, J. E., Neely, H., Rooney, W. L., Stupic, K. F., Weers, B., Zhu, B., Rosen, M. S., Morgan, C. L. S. (2020). Low-field magnetic resonance imaging of roots in intact clayey and silty soils. Geoderma, 370, 114356.

Bailey, V. L., Pries, C. H., & Lajtha, K. (2019). What do we know about soil carbon destabilization? Environmental Research Letters, 14(8), 083004.

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., & Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology, 57, 233–266.

Bakker, P. A. H. M., Berendsen, R. L., Doorhuis, R. F., Wintermans, P. C. A., & Pieterse, C. M. J. (2013). The rhizosphere revisited: Root microbiomics. Front. Plant Sci., 4, 165.

Bakker, M. G., Manter, D. K., Sheflin, A. M., Weir, T. L., & Vivanco, J. M. (2012). Harnessing the rhizosphere microbiome through plant breeding and agricultural management. Plant and Soil, 360(1–2), 1–13.

Balasundararajan, V., & Dananjayan, B. (2019). Occurrence of diversified N-acetyl homoserine lactone mediated biofilm-forming bacteria in rice rhizoplane. Journal of Basic Microbiology, 59(10), 1031–1039.

Barré, P., & Hallett, P. D. (2009). Rheological stabilization of wet soils by model root and fungal exudates depends on clay mineralogy. European Journal of Soil Science, 60(4), 525–538. https://doi.org/10.1111/j.1365-2389.2009.01151.x

Bates, T. R., & Lynch, J. P. (2000). Plant growth and phosphorus accumulation of wild type and two root hair mutants of Arabidopsis thaliana (Brassicaceae). American Journal of Botany, 87(7), 958–963.

Baudoin, E., Benizri, E., & Guckert, A. (2003). Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. Soil Biology and Biochemistry, 35(9), 1183–1192. https://doi.org/10.1016/s0038-0717(03)00179-2

Beckers, B., Op De Beeck, M., Weyens, N., Van Acker, R., Van Montagu, M., Boerjan, W., & Vangronsveld, J. (2016). Lignin engineering in field-grown poplar trees affects the endosphere bacterial microbiome. Proceedings National Academy of Science USA, 113(8), 2312–2317.

Bengough, A. G., Loades, K., & McKenzie, B. M. (2016). Root hairs aid soil penetration by anchoring the root surface to pore walls. Journal of Experimental Botany, 67(4), 1071–1078. https://doi.org/10.1093/jxbер/erw259

Berthelot, C., Chalot, M., Leyval, C., & Blaudez, D. (2019). From dark matter to light: Emergence of the mysterious dark septate endophytes in plant growth promotion and stress alleviation. In T. R. Hodkinson, F. M. Doohan, M. J. Saunders, & B. R. Murphy (Eds.), Endophytes for a growing world (pp. 143–164). Cambridge University Press. https://doi.org/10.1017/9781108607667.008

Bitterlich, M., Franken, P., & Graefe, J. (2018). Arbuscular mycorrhiza improves substrate hydraulic conductivity in the plant available moisture range under root growth exclusion. Frontiers in Plant Science, 9, 301.

Boddy, R. M., Urquiaga, S., Alves, B. J. R., & Reis, V. (2003). Endophytic nitrogen fixation in sugarcane: Present knowledge and future applications. Plant and Soil, 252(1), 139–149.

Boddy, E., Hill, P., Farrar, J., & Jones, D. (2007). Fast turnover of low molecular weight components of the dissolved organic carbon pool of temperate grassland field soils. Soil Biology and Biochemistry, 39(4), 827–835. https://doi.org/10.1016/j.soilbio.2006.09.030

Bogeat-Triboulot, M.-B., Brosché, M., Renaut, J., Jouve, L., Thiec, D. L.e, Fayyaz, P., Vinocur, B., Witters, E., Laukens, K., Teichmann, T.,
Altman, A., Hausman, J. F., Polle, A., Kangasjärvi, J., & Dreyer, E. (2007). Gradual soil water depletion results in reversible changes of gene expression, protein profiles, ecophysiology, and growth performance in Populus euphratica, a poplar growing in arid regions. *Plant Physiology, 143*(2), 876–892.

Bradford, M. A., Fierer, N., & Reynolds, J. F. (2008). Soil carbon stocks in experimental mesocosms are dependent on the rate of labile carbon, nitrogen and phosphorus inputs to soils. *Functional Ecology, 22*(6), 964–974. https://doi.org/10.1111/j.1365-2435.2008.01404.x

Bridgham, S. D., Cadillo-Quiroz, H., Keller, J. K., & Zhuang, Q. (2013). Methane emissions from wetlands: Biogeochemical, microbial, and modeling perspectives from local to global scales. *Global Change Biology, 19*(5), 1325–1346.

Brunner, I., Herzog, C., Galiano, L., & Gessler, A. (2019). Plasticity of fine-root traits under long-term irrigation of a water-limited scots pine forest. *Frontiers in Plant Science, 10*, 701.

Burak, E., Quinton, J. N., & Dodd, I. C. (2021). Root hairs are the most important root trait for rhizosheath formation of barley (*Hordeum vulgare*), maize (*Zea mays*) and Lotus japonicus (*Gifu*). *Annals of Botany, 128*(1), 45–57.

Burridge, J. D., Black, C. K., Nord, E. A., Postma, J. A., Sidhu, J. S., et al. (2020). An analysis of soil coring strategies to estimate root depth in maize and common bean. *Plant Phenomics, 2020*, 3252703.

Cai, G., Carminati, A., Abdalla, M., & Ahmed, M. A. (2021). Soil textures rather than root hairs dominate water uptake and soil–plant hydraulics under drought. *Plant Physiology*. https://doi.org/10.1093/plphys/kiab271

Canarini, A., Kaiser, C., Merchant, A., Richter, A., & Wanek, W. (2019). Root exudation of primary metabolites: Mechanisms and their roles in plant responses to environmental stimuli. *Frontiers in Plant Science, 10*. https://doi.org/10.3389/fpls.2019.00157

Canarini, A., Mariotte, P., Ingram, L., Merchant, A., & Dijkstra, F. A. (2018). Mineral-associated soil carbon is resistant to drought but sensitive to legumes and microbial biomass in an Australian grassland. *Ecosystems, 21*(2), 349–359. https://doi.org/10.1007/s10012-017-0152-x

Carminati, A., Passioua, J. B., Zarebanadkouki, M., Ahmed, M. A., Ryan, P. R., Watt, M., & Delhaize, E. (2017). Root hairs enable high transpiration rates in drying soils. *New Phytologist, 216*(3), 771–781.

Carthy, R. M., Mc Carthy, R., Löf, M., & Gardiner, E. S. (2018). Early root development of poplars (*Populus spp.*) in relation to moist and saturated soil conditions. *Scandinavian Journal of Forest Research, 33*(2), 125–132. https://doi.org/10.1080/02827581.2017.1338757

Carvalhais, L. C., Dennis, P. G., Fedoseyenko, D., Hajirezaei, M.-R., Borris, R., & Von Wirén, N. (2011). Root exudation of sugars, amino acids, and organic acids by maize as affected by nitrogen, phosphorus, potassium, and iron deficiency. *Journal of Plant Nutrition and Soil Science, 174*(1), 3–11. https://doi.org/10.1002/jpln.201000085

Cheng, W., & Coleman, D. C. (1990). Effect of living roots on soil organic matter decomposition. *Soil Biology and Biochemistry, 22*(6), 781–787. https://doi.org/10.1016/0038-0717(90)90157-u

Cheng, W., & Kuzyakov, Y. (2015). Root effects on soil organic matter decomposition. In R. W. Zobel & S. F. Wright (Eds.), *Roots and soil management: Interactions between roots and the soil* (Vol. 48, pp. 119–143). ASA, CSSA, and SSSA. https://doi.org/10.2134/agnromonogr48c7

Chhetri, H. B., Macaya-Sanz, D., Kainer, D., Biswal, A. K., Evans, L. M., Chen, J.-G., Collins, C., Hunt, K., Mohanty, S. S., Rosenstiel, T., Ryno, D., Winkeler, K., Yang, X., Jacobson, D., Mohnen, D., Muchero, W., Strauss, S. H., Tschaplinski, T. J., Tuskan, G. A., & Di Fazio, S. P. (2019). Multigene genome-wide association analysis of Populus trichocarpa identifies key polymorphisms controlling morphological and physiological traits. *New Phytol., 223*(1), 293–309.

Clemensen, A. K., Provenza, F. D., Hendrickson, J. R., & Grusak, M. A. (2020). Ecological implications of plant secondary metabolites: Phytochemical diversity can enhance agricultural sustainability. *Frontiers in Sustainable Food Systems, 4*, 233.

Clemente, J. S., Simpson, M. J., Simpson, A. J., Yanni, S. F., & Whalen, J. K. (2013). Comparison of soil organic matter composition after incubation with maize leaves, roots, and stems. *Geoderma, 192*, 86–96. https://doi.org/10.1016/j.geoderma.2012.08.007

Colombi, T., Herrmann, A. M., Vallenbach, P., & Keller, T. (2019). Cortical cell diameter is key to energy costs of root growth in wheat. *Plant Physiology, 180*(4), 2049–2060.

Comas, L. H., Becker, S. R., Cruz, V. M. V., Byrne, P. F., & Dierig, D. A. (2013). Root traits contributing to plant productivity under drought. *Front. Plant Sci.*, 4, 442.

Compant, S., Clement, C., & Sessitsch, A. (2010). Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology & Biochemistry, 42*(5), 669–678.

Cope, K. R., Irving, T. B., Chakraborty, S., & Ané, J.-M. (2021). Perception of lipo-chito-oligosaccharides by the bioenergy crop Populus. *Plant Signaling & Behavior, 16*(6), 1903758. https://doi.org/10.1080/1559222X.2021.1903758

Coskun, D., Britto, D. T., Shi, W., & Kronzucker, H. J. (2017). How plant root exudates shape the nitrogen cycle. *Trends in Plant Science, 22*(8), 661–673.

Cotrufo, M. F., Ranalli, M. G., Haddix, M. L., Six, J., & Lugato, E. (2019). Soil carbon storage informed by particulate and mineral-associated organic matter. *Nature Geoscience, 12*(12), 989–994.

Cruz, C., Green, J. J., Watson, C. A., Wilson, F., & Martins-Loução, M. A. (2004). Functional aspects of root architecture and mycorrhizal inoculation with respect to nutrient uptake capacity. *Mycorrhiza, 14*(3), 177–184.

Cunning, J. R., Zawaski, C., Desai, S., & Collart, F. R. (2015). Phosphorus disequilibrium in the tripartite plant-ectomycorrhiza-plant growth promoting rhizobacterial association. *Journal of Soil Science and Plant Nutrition, 15*(2). https://doi.org/10.4067/s0718-9562201505000040

Dakora, F. D., & Phillips, D. A. (2002). Root exudates as mediators of mineral acquisition in low-nutrient environments. In J. J. Adu-Gyamfi (Ed.), *Food security in nutrient-stressed environments: Exploiting plants’ genetic capabilities* (pp. 201–213). Springer. https://doi.org/10.1007/978-94-017-1570-6_23

Danjon, F., & Reubens, B. (2008). Assessing and analyzing 3D architecture of woody root systems, a review of methods and applications in tree and soil stability, resource acquisition and allocation. *Plant and Soil, 303*(1–2), 1–34.

Dash, M., Yordanov, Y. S., Georgieva, T., Tschaplinski, T. J., Yordanova, E., & Busov, E. (2017). Poplar PubZIP1-like enhances lateral root formation and biomass growth under drought stress. *Plant Journal, 89*(4), 692–705.

Dash, M., Yordanov, Y. S., Georgieva, T., Wei, H., & Busov, V. (2018). Gene network analysis of poplar root transcriptome in response to drought stress identifies a PtaJAZ3PtaRAP2.6-centered hierarchical network. *Plos One, 13*(12), e0208560.
De Deyn, G. B., Cornelissen, J. H. C., & Bardgett, R. D. (2008). Plant functional traits and soil carbon sequestration in contrasting biomes. *Ecology Letters, 11*(5), 516–531.

de Vries, F. T., Williams, A., Stringer, F., Willcocks, R., McEwing, R., Langridge, H., & Straathof, A. L. (2019). Changes in root-exudate-induced respiration reveal a novel mechanism through which drought affects ecosystem carbon cycling. *New Phytologist, 224*(1), 132–145.

Delhaize, E., Rathjen, T. M., & Cavanagh, C. R. (2015). The genetics of rhizosphere size in a multiparent mapping population of wheat. *Journal of Experimental Botany, 66*(15), 4527–4536. https://doi.org/10.1093/jxb/erv223

Desai, S., Naik, D., & Cumming, J. R. (2014). The influence of phosphorus availability and Laccaria bicolor symbiosis on phosphate acquisition, antioxidant enzyme activity, and rhizospheric carbon flux in *Populus tremuloides*. *Mycorrhiza, 24*(4), 369–382. https://doi.org/10.1007/s00572-013-0548-1

Di, T., Afzal, M. R., Yoshihashi, T., Deshpande, S., Zhu, Y., et al. (2018). Further insights into underlying mechanisms for the release of biological nitrification inhibitors from sorghum roots. *Plant and Soil, 423*(1–2), 99–110.

Ding, N., Huertas, R., Torres-Jerez, I., Liu, W., Watson, B., et al. (2021). Transcriptional, metabolic, physiological and developmental responses of switchgrass to phosphorus limitation. *Plant, Cell and Environment, 44*(1), 186–202.

Dini-Andreote, F. (2020). Endophytes: The second layer of plant defense. *Trends in Plant Science, 25*(4), 319–322.

Domenicano, S., Coll, L., Messier, C., & Berninger, F. (2011). Nitrogen forms affect root structure and water uptake in the hybrid poplar. *New Forests, 42*(3), 347–362. https://doi.org/10.1007/s11056-011-9256-x

Doty, S. L., Oakley, B., Xin, G., Kang, J. W., Singleton, G., et al. (2009). Diazotrophic endophytes of native black cottonwood and willow. *Symbiosis, 47*(1), 23–33.

Douglas, G. B., McVor, I. R., & Lloyd-West, C. M. (2016). Early root development of field-grown poplar: Effects of planting material and genotype. *New Zealand Journal of Forestry Science, 46*(1). https://doi.org/10.1186/s40490-015-0057-4

Dowd, T., McInturf, S., Li, M., & Topp, C. N. (2021). Rated-M for mesocosm: Allowing the multimodal analysis of mature root systems in 3D. *Emerging Topics in Life Sciences, 5*(2), 249–260.

Downie, H. F., Adu, M. O., Schmidt, S., Otten, W., Dupuy, L. X., White, P. J., & Valentine, T. A. (2015). Challenges and opportunities for quantifying roots and rhizosphere interactions through imaging and image analysis. *Plant, Cell and Environment, 38*(7), 1213–1232.

van Dusschoten, D., Metzner, R., Kochs, J., Postma, J. A., Pfugfelder, D., Bühler, J., Schurr, U., & Jahnke, S. (2016). Quantitative 3D analysis of plant roots growing in soil using magnetic resonance imaging. *Plant Physiology, 170*(3), 1176–1188.

Dynarski, K. A., Bossio, D. A., & Scow, K. M. (2020). Dynamic stability of soil carbon: Reassessing the “permanence” of soil carbon sequestration. *Frontiers of Environmental Science and Engineering, 8*, 218.

Egenolf, K., Verma, S., Schöne, J., Klaiber, I., Arango, J., Cadisch, G., Neumann, G., & Rasche, F. (2021). Rhizosphere pH and cation-anion balance determine the exudation of nitrification inhibitor 3-epibrachialactone suggesting release via secondary transport. *Physiologia Plantarum, 172*(1), 116–123.

Eilers, K. G., Lauber, C. L., Knight, R., & Fierer, N. (2010). Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. *Soil Biology and Biochemistry, 42*(6), 896–903. https://doi.org/10.1016/j.soilbio.2010.02.003

Ellie, O., Richter, R., Vohland, M., & Weigelt, A. (2019). Fine root lignin content is well predictable with near-infrared spectroscopy. *Scientific Reports, 9*(1), 6396.

Farooq, M., Wahid, A., Kobayashi, N., Fujiita, D., & Basra, S. M. A. (2009). Plant drought stress: Effects, mechanisms and management. In E. Lichtfouse, M. Navarrete, P. Debaeke, S. Véronique, & C. Alberola (Eds.), *Sustainable agriculture* (pp. 153–188). Springer.

Fierer, N., Breitbart, M., Nulton, J., Salamon, P., Lozupone, C., et al. (2007). Metagenomic and small-subunit rRNA analyses reveal the genetic diversity of bacteria, archaea, fungi, and viruses in soil. *Applied and Environmental Microbiology, 73*(21), 7059–7066.

Frank, A. B., Berdahl, J. D., Hanson, J. M., Liebig, A. M., & Johnson, H. A. (2004). Biomass and carbon partitioning in switchgrass. *Crop Science, 44*(4), 1391–1396. https://doi.org/10.2135/cropsci2004.1391

Freschet, G. T., Roumet, C., Comas, L. H., Weemstra, M., Bengough, A. G., Rewald, B., Bardgett, R. D., de Deyn, G. B., Johnson, D., Klimešová, J., Lukac, M., Mccormack, M. L., Meier, I. C., Pagés, L., Poorter, H., Prieto, I., Wurzburger, N., Zadworny, M., Bagñiewska-Zadworna, A., … Stokes, A. (2020). Root traits as drivers of plant and ecosystem functioning: Current understanding, pitfalls and future research needs. *New Phytologist, 232*(3), 1123–1158. https://doi.org/10.1111/nph.17072

Freschet, G. T., Pages, L., Iversen, C. M., Comas, L. H., Rewald, B., Roumet, C., Klimesova, J., Zadworny, M., Poorter, H., Postma, J. A., Adams, T. S., Bagñiewska-Zadworna, A., Bengough, A. G., Blancaflor, E. B., Brunner, I., Cornelissen, J. H. C., Garnier, E., Gessler, A., Hobbie, S. E., … McCormack M. L. (2021). A starting guide to root ecology: Strengthening ecological concepts and standardising root classification, sampling, processing and trait measurements. *New Phytologist, 232*, 973–1122.

Frey, S. D. (2019). Mycorrhizal fungi as mediators of soil organic matter dynamics. *Annual Review of Ecology, Evolution, and Systematics, 50*(1), 237–259.

Freycon, V., Wonkam, C., Fayolle, A., Laclau, J.-P., Lucot, E., et al. (2015). Tree roots can penetrate deeply in African semi-deciduous rainforests: Evidence from two common soil types. *Journal of Tropical Ecology, 31*(1), 13–23.

Fu, H. M., Meng, F. Y., Molatudi, R. L., & Zhang, B. G. (2016). Sorghum and switchgrass as biofuel feedstocks on marginal lands in northern China. *BioEnergy Research, 9*(2), 633–642. https://doi.org/10.1007/s12155-015-9704-0

Galindo-Castañeda, T., Brown, K. M., & Lynch, J. P. (2018). Reduced root cortical burden improves growth and grain yield under low phosphorus availability in maize. *Plant, Cell and Environment, 41*(7), 1579–1592.

Gargallo-Garriga, A., Preece, C., Sardans, J., Oravec, M., Urban, O., &Peruelas, J. (2018). Root exudate metabolomes change under drought and show limited capacity for recovery. *Scientific Reports, 8*(1), 12696.

Germon, A., Laclau, J.-P., Robin, A., & Jourdan, C. (2020). Tamm review: Deep fine roots in forest ecosystems: Why dig deeper? *Forest Ecology and Management, 466*, 118135.

Ghimire, S. R., Charlton, N. D., & Craven, K. D. (2009). The Mycorrhiza, *Mycorrhiza, 24*(4), 369–382. https://doi.org/10.1007/s00572-013-0548-1
Gomez-Casanovas, N., DeLucia, N. J., Hudiburg, T. W., Bernacchi, C. J., & DeLucia, E. H. (2018). Conversion of grazed pastures to energy cane as a biofuel feedstock alters the emission of GHGs from soils in Southeastern United States. *Biomass Bioenergy*, 108, 312–322.

Graaff, M.-A. D., de Graaff, M.-A., Six, J., Jastrow, J. D., Schadt, C. W., & Wullschleger, S. D. (2013). Variation in root architecture among switchgrass cultivars impacts root decomposition rates. *Soil Biology and Biochemistry*, 58, 198–206. https://doi.org/10.1016/j.soilbio.2012.11.015

Grandy, A. S., & Neff, J. C. (2008). Molecular C dynamics downstream: The biochemical decomposition sequence and its impact on soil organic matter structure and function. *The Science of the Total Environment*, 404(2–3), 297–307.

Gray, S. B., Streltner, R. S., Puthuvil, K. K., Ng, C., Shulman, R. E., et al. (2013). Minirhizotron imaging reveals that nodulation of field-grown soybean is enhanced by free-air CO2 enrichment only when combined with drought stress. *Functional Plant Biology*, 40(2), 137. https://doi.org/10.1071/fp12044

Griffiths, M., Roy, S., Guo, H., Seethapalli, A., Huhman, D., Ge, Y., Sharp, R. E., Fritschi, F. B., & York, L. M. (2021). A multiple ion-uptake phenotyping platform reveals shared mechanisms affecting nutrient uptake by roots. *Plant Physiology*, 185(3), 781–795.

Griffiths, M., Wang, X., Dhakal, K., Guo, H., & Seethapalli, A. (2021). Interactions among rooting traits for deep water and nitrogen uptake in upland and lowland ecotypes of switchgrass (*Panicum virgatum* L.). bioRxiv. https://www.biorxiv.org/content/10.1101/2021.02.19.432036v1.abstract

Griffiths, M., & York, L. M. (2020). Targeting root ion uptake kinetics to increase plant productivity and nutrient use efficiency. *Plant Physiology*, 182(4), 1854–1868.

Gschwendtner, S., Epscherschütz, J., Buegger, F., Reichmann, M., Müller, M., Schloter, M. (2011). Effects of genetically modified starch metabolism in potato plants on photosynthate fluxes into the rhizosphere and on microbial degraders of root exudates. *FEMS Microbiology Ecology*, 76(3), 564–575.

Guo, H., Ayalew, H., Seethapalli, A., Dhakal, K., Griffiths, M., Ma, X.-F., & York, L. M. (2021). Functional phenomics and genetics of the root economics space in winter wheat using high-throughput phenotyping of respiration and architecture. *New Phytologist*, 232(1), 98–112. https://doi.org/10.1111/nph.17329

Guo, L., Chen, J., Cui, X., Fan, B., & Lin, H. (2013). Application of ground penetrating radar for coarse root detection and quantification: A review. *Plant and Soil*, 362(1), 1–23.

Guo, H., & York, L. M. (2019). Maize with fewer nodal roots allocates mass to more lateral and deep roots that improve nitrogen uptake and shoot growth. *Journal of Experimental Botany*, 70(19), 5299–5309.

Guiter, J., Sawers, R. J. H., Marti, G., Andrés-Hernández, L., Yang, S.-Y., et al. (2015). Transcriptome diversity among rice root types during asymbiosis and interaction with arbuscular mycorrhizal fungi. *Proceedings of the National Academy of Sciences of the United States of America*, 112(21), 6754–6759.

Guyonnet, J. P., Vautrin, F., Meiffren, G., Labois, C., Cantarel, A. A. M., Michalet, M., Comte, G., Haichar, F. E. Z. (2017). The effects of plant nutritional strategy on soil microbial denitrification activity through rhizosphere primary metabolites. *FEMS Microbiology Ecology*, 93(4). https://doi.org/10.1093/femsec/fix022

Haichar, F. E. Z., Marol, C., Berge, O., Rangel-Castro, J. I., Prosser, J. I., Balesdent, J., Heulin, T., & Achouak, W. (2008). Plant host habitat and root exudates shape soil bacterial community structure. *ISME Journal*, 2(12), 1221–1230.

Haling, R. E., Brown, L. K., Bengough, A. G., Valentine, T. A., White, P. J., Young, I. M., & George, T. S. (2014). Root hair length and rhizosphere mass depend on soil porosity, strength and water content in barley genotypes. *Planta*, 239(3), 643–651.

Haling, R. E., Brown, L. K., Bengough, A. G., Young, I. M., Hallett, P. D., White, P. J., & George, T. S. (2013). Root hairs improve root penetration, root–soil contact, and phosphorus acquisition in soils of different strength. *Journal of Experimental Botany*, 64(12), 3711–3721.

Haling, R. E., Yang, Z., Shadwell, N., Culvenor, R. A., Stefanski, A., Ryan, M. H., Sandral, G. A., Kidd, D. R., Lambers, H., & Simpson, R. J. (2016). Root morphological traits that determine phosphorus-acquisition efficiency and critical external phosphorus requirement in pasture species. *Functional Plant Biology*, 43(9), 815–826.

Hamanishi, E. T., Barchet, G. L. H., Dauwe, R., Mansfield, S. D., & Campbell, M. M. (2015). Poplar trees reconfigure the transcriptome and metabolome in response to drought in a genotype- and time-of-day-dependent manner. *BMC Genomics [Electronic Resource]*, 16(1). https://doi.org/10.1186/s12864-015-1535-z

Hamanishi, E. T., Raj, S., Wilkins, O., Thomas, B. R., Mansfield, S. D., & Campbell, M. M. (2010). Intraspecific variation in the *Populus balsamifera* drought transcriptome. *Plant, Cell and Environment*, 33(10), 1742–1755.

Hartman, K., & Tringe, S. G. (2019). Interactions between plants and soil shaping the root microbiome under abiotic stress. *Biochemical Journal*, 476(19), 2705–2724.

Henry, S., Texier, S., Hallet, S., Bru, D., Dambreville, C., Chênevy, D., Bizouard, F., Germon, J. C., & Philipott, L. (2008). Disentangling the rhizosphere effect on nitrate reducers and denitrifiers: Insight into the role of root exudates. *Environmental Microbiology*, 10(11), 3082–3092.

Higa, A., Mori, Y., & Kitamura, Y. (2010). Iron deficiency induces changes in riboflavin secretion and the mitochondrial electron transport chain in hairy roots of *Hyoscyamus albus*. *Journal of Plant Physiology*, 167(11), 870–878.

Hochman, G., & Zilberman, D. (2018). Corn ethanol and U.S. biofuel policy 10 years later: A quantitative assessment. *American Journal of Agricultural Economics*, 100(2), 570–584.

Hodge, A. (2004). The plastic plant: Root responses to heterogeneous supplies of nutrients. *New Phytologist*, 162(1), 9–24. https://doi.org/10.1111/j.1469-8137.2004.01015.x

Hodge, A. (2009). Root decisions. *Plant, Cell and Environment*, 32(6), 628–640.

Inderjit, I., & Weston, L. A. (2003). Root Exudates: An Overview. *Root Ecology*, 235–255. https://doi.org/10.1007/978-3-662-09784-7_10

Intergovernmental Panel on Climate Change. (2021). *Climate Change 2021: The physical science basis*. Cambridge University Press.

Iversen, C. M., McCormack, M. L., Powell, A. S., Blackwood, C. B., Freschet, G. T., et al. (2017). A global fine-root ecology database to address below-ground challenges in plant ecology. *New Phytologist*, 215(1), 15–26.

Jackson, L. E., Bowles, T. M., Hodson, A. K., & Lazcano, C. (2012). Soil microbial-root and microbial-rhizosphere processes to increase nitrogen availability and retention in agroecosystems. *Current Opinion in Environmental Sustainability*, 4(5), 517–522.
Jackson, R. B., Canadell, J., Ehleringer, J. R., Mooney, H. A., Sala, O. E., & Schulze, E. D. (1996). A global analysis of root distributions for terrestrial biomes. *Oecologia*, 108(3), 389–411.

James, R. A., Welgama, C., Verbyla, K., Ryan, P. R., Rebetzke, G. J., Rattey, A., Richardson, A. E., & Delhaize, E. (2016). Rhizosheaths on wheat grown in acid soils: Phosphorus acquisition efficiency and genetic control. *Journal of Experimental Botany*, 67(12), 3709–3718.

Jansson, J. K., & Hofmockel, K. S. (2018). The soil microbiome–from metagenomics to metatransomics. *Current Opinion in Microbiology*, 43, 162–168.

Jiang, F., Zhang, L., Zhou, J., George, T. S., & Feng, G. (2021). Arbuscular mycorrhizal fungi enhance mineralisation of organic phosphorus by carrying bacteria along their extra-radical hyphae. *New Phytologist*, 230(1), 304–315.

Kameoka, E., Suralta, R. R., Mitsuya, S., & Yamauchi, A. (2015). Matching the expression of root plasticity with soil moisture availability maximizes production of rice plants grown in an experimental sloping bed having soil moisture gradients. *Plant Production Science*, 18(3), 267–276. https://doi.org/10.1626/pps.18.267

Karwat, H., Moreta, D., Arango, J., Núñez, J., Rao, I., et al. (2017). Residual effect of BNI by Bracharia humidicola pastures on nitrogen recovery and grain yield of subsequent maize. *Plant and Soil*, 420(1–2), 389–406.

Kattge, J., Bönisch, G., Díaz, S., Lavorel, S., Prentice, I. C., Leadley, P., Tautenhahn, S., Werner, G. D. A., Aakala, T., Abedi, M., Acosta, A. T. R., Adaminis, G. C., Adamson, K., Aiba, M., Albert, C. H., Alcántara, J. M., Alcázar, C., Aleixo, I., Ali, H., … Wirth, C. (2020). TRY plant trait database: Enhanced coverage and open access. *Global Change Biology*, 26(1), 119–188.

Kell, D. B. (2011). Breeding crop plants with deep roots: Their role in sustainable carbon, nutrient and water sequestration. *Annals of Botany*, 108(3), 407.

Kewyn, D. P., & Kent, A. D. (2014). Contribution of nitrogen fixation to first year Miscanthus × giganteus. *Global Change Biology Bioenergy*, 6(5), 577–586.

Kim, D., Lee, M.-I., & Seo, E. (2019). Improvement of soil respiration parameterization in a dynamic global vegetation model and its impact on the simulation of terrestrial carbon fluxes. *Journal of Climate*, 32(1), 127–143.

Kirfel, K., Leuschner, C., Hertel, D., & Schulte, B. (2017). Influence of root diameter and soil depth on the xylem anatomy of fine- to medium-sized roots of mature beech trees in the top- and subsoil. *Frontiers in Plant Science*, 8, 1194.

Kleber, M., & Johnson, M. G. (2010). Advances in understanding the molecular structure of soil organic matter. In D. L. Sparks (Ed.), *Advances in agronomy* (Vol. 106, pp. 77–142). Elsevier. https://doi.org/10.1016/s0065-2113(10)60033-7

Kleidon, A., & Heimann, M. (2000). Assessing the role of deep rooted vegetation in the climate system with model simulations: Mechanism, comparison to observations and implications for Amazonian deforestation. *Climate Dynamics*, 16(2–3), 183–199.

Klein, S. P., Schneider, H. M., Perkins, A. C., Brown, K. M., & Lynch, J. P. (2020). Multiple integrated root phenotypes are associated with improved drought tolerance. *Plant Physiology*, 183(3), 1011–1025.

Klugh, K. R., & Cumming, J. R. (2007). Variations in organic acid exudation and aluminium resistance among arbuscular mycorrhizal species colonizing Liriodendron tulipifera. *Tree Physiology*, 27(8), 1103–1112.

Klugh-Stewart, K., & Cumming, J. R. (2009). Organic acid exudation by mycorrhizal *Andropogon virginicus* L. (bromegrass) roots in response to aluminum. *Soil Biology and Biochemistry*, 41(2), 367–373. https://doi.org/10.1016/j.soilbio.2008.11.013

Kong, A. Y. Y., & Six, J. (2010). Tracing root vs. residue carbon into soils from conventional and alternative cropping systems. *Soil Science Society of America Journal*, 74(4), 1201–1210. https://doi.org/10.2136/ssaj2009.0346

Korenblum, E., Dong, Y., Szymanski, J., Panda, S., Jozwiak, A., Musalla, H., Meir, S., Rogachev, I., & Aharoni, A. (2020). Rhizosphere microbiome mediates systemic root metabolite exudation by root-to-root signalling. *PNAS*, 117, 3874–3883.

Krafczyk, I., Trolldenier, G., & Beringer, H. (1984). Soluble root exudates of maize: Influence of potassium supply and rhizosphere microorganisms. *Soil Biology & Biochemistry*, 16(4), 315–322.

Kumar, A., & Singh, J. (2020). Biofilms forming microbes: Diversity and potential application in plant–microbe interaction and plant growth. In A. Yadav, J. Singh, A. Rastegari, & N. Yadav (Eds.), *Plant microbomes for sustainable agriculture: Vol. 25. Sustainable development and biodiversity* (pp. 173–197). Springer. https://doi.org/10.1007/978-3-030-38453-1_6

Kuziyakov, Y., Friedel, J. K., & Stahr, K. (2000). Review of mechanisms and quantification of priming effects. *Soil Biology and Biochemistry*, 32(11–12), 1485–1498. https://doi.org/10.1016/s0038-0717(00)00084-5

Labbé, J., Muchero, W., Czarnecki, O., Wang, J., Wang, X., Bryan, A. C., Zheng, K., Yang, Y., Xie, M., Zhang, J., Wang, D., Meidl, P., Wang, H., Morrell-Falvey, J. L., Copé, K. R., Maia, L. G. S., Ané, J. M., Mewalal, R., Jawdy, S. S., … Tuskan, G. A. (2019). Mediation of plant–mycorrhizal interaction by a lectin receptor-like kinase. *Nature Plants*, 5(7), 676–680.

Lawson, C. E., Harcombe, W. R., Hatzenpichler, R., Lindemann, S. R., Löfler, F. E., O’Malley, M. A., Martin, H. G., Pfleger, B. F., Raskin, L., Ophelia, S., Venturelli, O. S., Weissbrodt, D. G., Noguera, D. R., & McMahon, K. D. (2019). Common principles and best practices for engineering microbiomes. *Nature Reviews Microbiology*, 17(12), 725–741.

Lawton, J. H., Naeem, S., Woodfin, R. M., Brown, V. K., Gange, A., Godfray, H. J. C., Heads, P. A., Lawler, S., Magda, D., Thomas, C. D., Thompson, L. J., & Young, S. (1993). The Ecotron: A controlled environmental facility for the investigation of population and ecosystem processes. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 341(1296), 181–194. https://doi.org/10.1098/rstb.1993.0102

Legner, N., Meinen, C., & Rauber, R. (2018). Root Differentiation of agricultural plant cultivars and progeniences using FTIR spectroscopy. *Frontiers in Plant Sciences*, 9, 748.

Li, H., Böltscher, T., Winnick, M., Tially, M. M., Cardon, Z. G., & Keiluweit, M. (2021). Simple plant and microbial exudates destabilize mineral-associated organic matter via multiple pathways. *Environmental Science & Technology*, 55(5), 3389–3398.

Li, Y., Li, Q., Guan, G., & Chen, S. (2020). Phosphate solubilizing bacteria stimulate wheat rhizosphere and endosphere biological nitrogen fixation by improving phosphorus content. *PeerJ*, 8, e9062.

Lin, C.-Y., Vuu, K. M., Amer, B., Shih, P. M., Baidoo, E. E. K., Scheller, H. V., & Eudes, A. (2021). In planta production of the
biodegradable polyester precursor 2-pyrone-4,6-dicarboxylic acid (PDC): Stacking reduced biomass recalcitrance with value-added co-product. Metabolic Engineering, 66, 148–156. https://doi.org/10.1016/j.menb.2021.04.011

Liu, T.-Y., Chen, M.-X., Zhang, Y., Zhu, F.-Y., Liu, Y.-G., Tian, Y., Fernie, A. R., Ye, N. & Zhang, J. (2019). Comparative metabolite profiling of two switchgrass ecotypes reveals differences in drought stress responses and rhizosphere weight. Planta, 250(4), 1355–1369.

Liu, X., Dong, X., Xue, Q., Leskovar, D. I., Jifon, J., Butnor, J. R., & Marek, T. (2018). Ground penetrating radar (GPR) detects fine roots of agricultural crops in the field. Plant and Soil, 423(1-2), 517–531.

Liu, T.-Y., Ye, N., Song, T., Cao, Y., Gao, B., Zhang, D., Zhu, F., Chen, M., Zhang, Y., Xu, W., & Zhang, J. (2019). Rhizosphere formation and involvement in foxtail millet (Setaria italica) root growth under drought stress. Journal of Integrative Plant Biology, 61(4), 449–462.

Lobet, G., Draye, X., & Périlleux, C. (2013). An online database for plant image analysis software tools. Plant Methods, 9(1), 38.

Lobet, G., Pagès, L., & Draye, X. (2011). A novel image-analysis tool-box enabling quantitative analysis of root system architecture. Plant Physiology, 157(1), 29–39.

Lynch, J. (1995). Root architecture and plant productivity. Plant Ecology, 109(1), 7–13. https://doi.org/10.1040/1109.1.7

Lynch, J. P. (2019). Root phenotypes for improved nutrient capture: An underexploited opportunity for global agriculture. New Phytologist, 223(2), 548–564.

Lynch, J. P., Chimungu, J. G., & Brown, K. M. (2014). Root anatomical phenes associated with water acquisition from drying soil: Targets for crop improvement. Journal of Experimental Botany, 65(21), 6155–6166.

Ma, J. F., Goto, S., Tamai, K., & Ichii, M. (2001). Role of root hairs and lateral roots in silicon uptake by rice. Plant Physiology, 127(4), 1773–1780.

Martin-Rivilla, H., Garcia-Villarco, A., Ramos-Solano, B., Gutierrez-Lobet, G., Pagès, L., & Draye, X. (2011). A novel image-analysis tool-box enabling quantitative analysis of root system architecture. Plant Physiology, 157(1), 29–39.

O'Toole, J. C., & Bland, W. L. (1987). Genotypic variation in crop root systems. In N. C. Brady (Ed.), Advances in agronomy (Vol. 41, pp. 91–145). Elsevier. https://doi.org/10.1016/s0065-2113(08)60803-2

Pace, J., Lee, N., Naik, H. S., Ganapathysubramanian, B., & Lübberstedt, T. (2014). Analysis of maize (Zea mays L.) seedling roots with the high-throughput image analysis tool ARIA (Automatic Root Image Analysis). Plos One, 9(9), e108255.

Paliiy, O., & Shankar, V. (2016). Application of multivariate statistical techniques in microbial ecology. Molecular Ecology, 25(5), 1032–1057.

Peruzzo, L., Chou, C., Wu, Y., Schmutz, M., Mary, B., Wagner, F. M., Petrov, P., Newman, G., Blancaflor, E. B., & Monteros, M. J. (2015). Root traits and phenotyping strategies for plant improvement. Plants, 4(2), 334–355.

Pierret, A., Maeght, J.-L., Clément, C., Montoroi, J.-P., Hartmann, C., & Gonkhamdee, S. (2016). Understanding deep roots and their functions in ecosystems: An advocacy for more unconventional research. Annals of Botany, 118(4), 621–635.
Plett, J. M., Daguerre, Y., Wittulsky, S., Vayssières, A., Deveau, A., et al. (2014). Effector MiSFP7 of the mutualistic fungus Laccaria bicolor stabilizes the Populus JAZs protein and represses Jasmonic acid (JA) responsive genes. *Proceedings of the National Academy of Sciences of the United States of America*, 111(22), 8299–8304.

Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., et al. (2012). Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control. *New Phytologist*, 193(1), 30–50. https://doi.org/10.1111/j.1469-8137.2011.03952.x

Popović, B. M., Štajner, D., Ždro-Pavlović, R., Tumbas-Saponjic, V., Čanadanović-Brunet, J., & Orlović, S. (2016). Water stress induces changes in polyphenol profile and antioxidant capacity in poplar plants (*Populus* spp.). *Plant Physiology and Biochemistry*, 105, 242–250.

Pound, M. P., French, A. P., Atkinson, J. A., Wells, D. M., Bennett, M. J., & Pridmore, T. (2013). RootNav: Navigating images of complex root architectures. *Plant Physiology*, 162(4), 1802–1814.

Preece, C., & Peñuelas, J. (2016). Rhizodeposition under drought and consequences for soil communities and ecosystem resilience. *Plant and Soil*, 409(1-2), 1–17. https://doi.org/10.1007/s11104-016-3090-z

Pregitzer, K. S., DeForest, J. L., Burton, A. J. Allen, M. F., Ruess, R. W., & Hendrick, R. L. (2004). Fine root architecture of nine northern American trees. *Ecological Monographs*, 72(2), 293–309. https://doi.org/10.1890/0012-9615(2002)072[0293:faarno.2.co.2]

Provorov, N. A. (2009). Plant-microbe symbioses as an evolutionary continuum. *Journal of Plant Ecology*, 27(1), 10–34.

Quinn, L. D., Straker, K. C., Guo, J., Kim, S., Thapa, S., Kling, G., Lee, D. K., & Voigt, T. B. (2015). Stress-tolerant feedstocks for sustainable bioenergy production on marginal land. *Bioenergy Research*, 8(3), 1081–1100.

Rasse, D. P., Rumpel, C., & Dignac, M.-F. (2005). Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant and Soil*, 269(1–2), 341–356. https://doi.org/10.1007/s11104-004-0907-y

Regier, N., Streb, S., Cocozza, C., Schaub, M., Cherubini, P., Zeeman, S. Z., & Frey, B. (2009). Drought tolerance of two black poplar (*Populus nigra* L.) clones: Contribution of carbohydrates and oxidative stress defence. *Plant, Cell and Environment*, 32(12), 1724–1736.

Rencoret, J., Manques, G., Serrano, O., Kaal, J., Martinez, A. T., del Río, J. C., & Gutiérrez, A. (2020). Deciphering the unique structure and acylation pattern of *Posidonia oceanica* lignin. *ACS Sustainable Chemistry & Engineering*, 8(33), 12521–12533. https://doi.org/10.1021/acssuschemeng.0c03502

Robertson, G. P., Hamilton, S. K., Barham, B. L., Dale, B. E., Izaurralde, R. C., Jackson, R. D., Landiss, D. A., Swinton, S. M., Thelen, K. D., & Tiedje, J. M. (2017). Cellulosic biofuel contributions to a sustainable energy future: Choices and outcomes. *Science*, 356(6345). https://doi.org/10.1126/science.aal2324

Rolfé, S. A., Griffiths, J., & Ton, J. (2019). Crying out for help with root exudates: Adaptive mechanisms by which stressed plants assemble health-promoting soil microorganisms. *Current Opinion in Microbiology*, 49, 73–82.

Rose, L. (2017). Pitfalls in root trait calculations: How ignoring diameter heterogeneity can lead to overestimation of functional traits. *Frontier in Plant Science*, 8, 898.

Ruiz, S., Koebnick, N., Duncan, S., McKay Fletcher, D., & Scotson, C., Boghi, A., Marin, M., Bengough, A. G., George, T. S., Brown, L. K., Hallett, P. D., & Roose, T. (2020). Significance of root hairs at the field scale: Modelling root water and phosphorus uptake under different field conditions. *Plant and Soil*, 447(1–2), 281–304. https://doi.org/10.1007/s11104-019-04308-2

Rush, T. A., Puech-Pagés, V., Bascuales, A., Jargeat, P., Mailet, F., Haouy, A., Maës, A. Q., Carriel, C. C., Khokhani, D., Keller-Pearson, M. R., Tannous, J., cope, K. R., Garcia, K., Maeda, J., Johnson, C., Kleven, B., Choudhury, Q. J., Labbé, J., Swift, C., ... Ané, J. M. (2020). Lipo-chito-oligosaccharides as regulatory signals of fungal growth and development. *Nature Communications*, 11(1), 3897.

Ryan, M. G., & Law, B. E. (2005). Interpreting, measuring, and modeling soil respiration. *Biogeochemistry*, 73(1), 3–27. https://doi.org/10.1007/s10533-004-5167-7

Salas-González, I., Reyt, G., Flis, P., Custódio, V., Gopaluchan, D., Bakhoun, N., Dew, T. P., Suresh, K., Franke, R. B., Dangl, J. L., Salt, D. E., & Castrillo, G. (2021). Coordination between microbiota and root endosperm supports plant mineral nutrient homeostasis. *Science*, 371(6525). https://doi.org/10.1126/science.abc0695

Saleem, M., Law, A. D., Sahib, M. R., Pervaiz, Z. H., & Zhang, Q. (2018). Impact of root system architecture on rhizosphere and root microbiome. *Rhizosphere*, 6, 47–51.

Sasse, J., Kant, J., Cole, B. J., Klein, A. P., Arsova, B., Schlaepfer, P., Gao, J., Lewald, K., Zhalnina, K., Kosina, S., Bowen, B. P., Treen, D., Vogel, J., Visel, A., Watt, M., Dangl, J. L., & Northen, T. R. (2019). Multilab EcoFAB study shows highly reproducible physiology and depletion of soil metabolites by a model grass. *New Phytologist*, 222(2), 1149–1160.

Sasse, J., Kosina, S. M., Raad, M., Jordan, J. S., Whiting, K., Zhalnina, K., & Northen, T. R. (2020). Root morphology and exudate availability are shaped by particle size and chemistry in Brachypodium distachyon. *Plant Direct*, 4(7). https://doi.org/10.1002/pld3.207

Sasse, J., Martinoia, E., & Northen, T. (2018). Feed your friends: Do plant exudates shape the root microbiome? *Trends in Plant Science*, 23(1), 25–41.

Sattelmacher, B. (2001). The apoplastic and its significance for plant mineral nutrition. *New Phytologist*, 149(2), 167–192.

Schetter, A., Lin, C.-H., Zumpf, C., Jiang, C., Hoffmann, L., Rooney, W., & Lee, D. K. (2021). Genotype–environment–management interactions in biomass yield and feedstock composition of photoperiod-sensitive energy sorghum. *BioEnergy Research*. https://doi.org/10.1007/s12155-021-10272-6

Schmidt, M. W. I., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D. A. C., Nannipieri, P., Rasse, D. P., Weiner, S., & Trumbore, S. E. (2011). Persistence of soil organic matter as an ecosystem property. *Nature*, 478(7367), 49–56.

Schneider, H. M., & Lynch, J. P. (2020). Should Root Plasticity Be a Crop Breeding Target? *Frontiers in Plant Science*, 11, 546.

Seethapalli, A., Dhakal, K., Griffiths, M., Guo, H., Freschet, G. T., & York, L. M. (2021). RhizoVision Explorer: Open-source software for root image analysis and measurement standardization. *AoB PLANTS*. https://doi.org/10.1093/aobpla/plab056

Seethapalli, A., Guo, H., Liu, X., Griffiths, M., Almatarfi, H., Li, Z., Liu, S., Zare, A., Fritschi, F. B., Blancaflor, E. B., Ma, X.-F., & York, L. M. (2020). RhizoVision Crown: An integrated hardware and software platform for root crown phenotyping. *Plant Phenomics*, 2020, 3074916.

Shinde, S., Cumming, J. R., Collart, F. R., Noiroit, P. H., & Larsen, P. E. (2017). Transportome Is Linked to Strain-Specific Plant Growth Promotion in Aspen Seedlings under nutrient stress. *Frontiers in Plant Science*, 8, 348.
Shinde, S., Zerbs, S., Collart, F. R., Cumming, J. R., Noiroit, P., & Larsen, P. E. (2019). Pseudomonas fluorescens increases mycorrhization and modulates expression of antifungal defense response genes in roots of aspen seedlings. *BMC Plant Biology [Electronic Resource]*, 19(1), 4.

Shi, S., Richardson, A. E., O’Callaghan, M., DeAngelis, K. M., Jones, E. E., Stewart, A., Firestone, M. K., & Condon, L. M. (2011). Effects of selected root exudate components on soil bacterial communities. *FEMS Microbiology Ecology*, 77(3), 600–610.

Silver, W. L., & Miya, R. K. (2001). Global patterns in root decomposition: Comparisons of climate and litter quality effects. *Oecologia*, 129(3), 407–419. https://doi.org/10.1007/s004420000740

Simon, P. L., de Klein, C. A. M., Worth, W., Rutherford, A. J., & Dieckow, J. (2019). The efficacy of *Plantago lanceolata* for mitigating nitrous oxide emissions from cattle urine patches. *The Science of the Total Environment*, 691, 430–441.

Simpson, A. J., Simpson, M. J., Smith, E., & Kelleher, B. P. (2007). Microbially derived inputs to soil organic matter: Are current estimates too low? *Environmental Science & Technology*, 41(23), 8070–8076.

Singer, E., Vogel, J. P., Northen, T., Mungall, C. J., & Juenger, T. E. (2021). Novel and emerging capabilities that can provide a holistic understanding of the plant root microbiome. *Phytobiomes Journal: PBIOMES—05*. https://doi.org/10.1094/phoimes-05-20-0042-rvw

Smith, A. G., Han, E., Petersen, J., Olsen, N. A. F., & Giese, C. (2020). RootPainter: Deep learning segmentation of biological images with corrective annotation. *BioRxiv*. https://www.biorxiv.org/content/10.1101/2020.04.16.044461v2.abstract

Smith, S. E., & Read, D. J. (1996). *Mycorrhizal symbiosis*. Elsevier.

Sun, Y., Qiao, Z., Muchero, W., & Chen, J.-G. (2020). Lectin receptor-like kinases: The sensor and mediator at the plant cell surface. *Frontiers in Plant Science*, 11. https://doi.org/10.3389/fpls.2020.596301

Tang, C., Li, S., Li, M., & Xie, G. H. (2018). Bioethanol potential of energy sorghum grown on marginal and arable lands. *Frontiers in Plant Science*, 9, 440.

Teeri, J. A., & Raven, P. H. (2002). A national ecological observatory network. *Science*, 298(5600), 1893.

Topp, C. N., Bray, A. L., Ellis, N. A. F., & Liu, Z. (2016). How can we harness quantitative genetic variation in crop root systems for agricultural improvement? *Journal of Integrative Plant Biology*, 58(3), 213–225.

Torres, M. S., White, J. F., Zhang, X., Hinton, D. M., & Bacon, C. W. (2012). Endophyte-mediated adjustments in host morphology and physiology and effects on host fitness traits in grasses. *Fungal Ecology*, 5(3), 322–330.

Trachsel, S., Kaeppler, S. M., Brown, K. M., & Lynch, J. P. (2011). Shovelomics: High throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. *Plant and Soil*, 341(1–2), 75–87. https://doi.org/10.1007/s11104-010-0623-8

Tracy, S. R., Nagel, K. A., Postma, J. A., Fassbender, H., Wasson, A., & Watt, M. (2020). Crop improvement from phenotyping roots: Highlights reveal expanding opportunities. *Trends in Plant Science*, 25(1), 105–118.

Tracy, S. R., Roberts, J. A., Black, C. R., McNeeil, A., Davidson, R., & Mooney, S. J. (2010). The X-factor: Visualizing undisturbed root architecture in soils using X-ray computed tomography. *Journal of Experimental Botany*, 61(2), 311–313.

Tschaplinski, T. J., Abraham, P. E., Jawdy, S. S., Gunter, L. E., Martin, M. Z., Engle, N. L., Yang, X., & Tuskan, G. A. (2019). The nature of the progression of drought stress drives differential metabolic responses in *Populus deltoides*. *Annals of Botany*, 124(4), 617–626.

Tyree, M. T., Davis, S. D., & Cochard, H. (1994). Biophysical perspectives of xylem evolution: Is there a tradeoff of hydraulic efficiency for vulnerability to dysfunction? *IWA Journal*, 15(4), 335–360.

Uren, N. (2007). Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants. In R. Pinton, Z. Varanini, & P. Nannipieri (Eds.), *The rhizosphere: Biochemistry and organic substances at the soil—plant interface*. (pp. 1–21). CRC Press.

Villegas, D., Arevalo, A., Nuñez, J., Mazabel, J., Subbarao, G., Rao, I., de Vega, J., & Arango, J. (2020). Biological nitrification inhibition (BNI): Phenotyping of a core germplasm collection of the tropical forage grass under greenhouse conditions. *Frontiers in Plant Science*, 11, 820.

von Harden, A. C., Kucharik, C. J., Jackson, R. D., & Marin-Spiotta, E. (2019). Litter quantity, litter chemistry, and soil texture control changes in soil organic carbon fractions under bioenergy cropping systems of the *North Central U.S. Biogeochecology*, 143(3), 313–326.

Vranova, V., Rejsek, K., Skene, K. R., Janous, D., & Formanek, P. (2013). Methods of collection of plant root exudates in relation to plant metabolism and purpose: A review. *Journal of Plant Nutrition and Soil Science*, 176(2), 175–199. https://doi.org/10.1002/jpln.201000360

Wahid, P. A. (2000). A system of classification of woody perennials based on their root activity patterns. *Agroforestry Systems*, 49(2), 123–130.

Wang, F., & Feng, G. (2021). Arbuscular mycorrhizal fungi interactions in the rhizosphere. In V. S. R. Gupta & A. K. Sharma (Eds.), *Rhizosphere biology: Interactions between microbes and plants.*: 217–235. https://doi.org/10.1007/978-91-56125-2_11

Tang, W., Rostamzadeh, M., Song, Z., Wang, L., McNickle, G., et al. (2019). SegRoot: A high throughput segmentation method for root image analysis. *Computers and Electronics in Agriculture*, 162, 845–854.

Wang, B., Seiler, J. R., & Mei, C. (2016). A microbial endophyte enhanced growth of switchgrass under two drought cycles improving leaf level physiology and leaf development. *Environmental and Experimental Botany*, 122, 100–108. https://doi.org/10.1016/j.envexpbot.2015.09.004

Watt, M., McCully, M. E., & Canney, M. J. (1994). Formation and stabilization of rhizosheaths of *Zea mays* L. (effect of soil water content). *Plant Physiology*, 106(1), 179–186.

Weigelt, A., Mommer, L., Andraczek, K., Iversen, C. M., Bergmann, J., Bruehlbeide, H., Fan, Y., Freschet, G. T., Guerrero-Ramírez, N. R., Kattge, J., Kuyper, T. W., Laughlin, D. C., Meier, I. C., van der Plas, F., Poorter, H., Roumet, C., van Ruijven, J., Sabatini, F. M., Semchenko, M., … McCormack, M. L. (2021). An integrated framework of plant form and function: The belowground perspective. *New Phytologist*. https://doi.org/10.1111/nph.17590

Wieder, W. R., Boehnert, J., & Bonan, G. B. (2014). Evaluating soil biogeochemistry parameterizations in Earth system models with observations. *Global Biogeochemical Cycles*, 28(3), 211–222. https://doi.org/10.1002/gbc.2004665

Wu, R., Grissom, J. J., McKeand, S. E., & O’Malley, D. M. (2004). Phenotypic plasticity of fine root growth increases plant productivity in pine seedlings. *BMC Ecology [Electronic Resource]*, 4, 14.

Xu, X., Sun, Y., Sun, J., Cao, P., Wang, Y., Chen, H. Y. H., Wang, W., & Ruan, H. (2020). Cellulose dominantly affects soil fauna in the decomposition of forest litter: A meta-analysis. *Geoderma*, 378, 114620.
Yang, P., Cai, X., & Khanna, M. (2021). Farmers’ heterogeneous perceptions of marginal land for biofuel crops in US Midwestern states considering biophysical and socioeconomic factors. *Global Change Biology Bioenergy, 13*(5), 849–861.

Yang, Y., & Tilman, D. (2020). Soil and root carbon storage is key to climate benefits of bioenergy crops. *Biofuel Research Journal, 7*(2), 1143–1148. https://doi.org/10.18331/brj2020.7.2.2

Yan, X.-L., Jia, L., & Dai, T. (2019). Fine root morphology and growth in response to nitrogen addition through drip fertigation in a *Populus × euramericana* “Guariento” plantation over multiple years. *Annals of Forest Science, 76*(1). https://doi.org/10.1007/s13595-019-0798-y

York, L. M. (2019). Functional phenomics: An emerging field integrating high-throughput phenotyping, physiology, and bioinformatics. *Journal of Experimental Botany, 70*(2), 379–386.

York, L. M., Carminati, A., Mooney, S. J., Ritz, K., & Bennett, M. J. (2016). The holistic rhizosphere: Integrating zones, processes, and semantics in the soil influenced by roots. *Journal of Experimental Botany, 67*(12), 3629–3643.

York, L. M., & Lynch, J. P. (2015). Intensive field phenotyping of maize (*Zea mays L.*) root crowns identifies phenes and phene integration associated with plant growth and nitrogen acquisition. *Journal of Experimental Botany, 66*(18), 5493–5505.

York, L. M., Nord, E. A., & Lynch, J. P. (2013). Integration of root phenes for soil resource acquisition. *Frontiers in Plant Science, 4*, 355.

Yu, F., Liang, K., Han, X., Du, D., Pan, Z., & Qiu, F. (2019). Major natural genetic variation contributes to waterlogging tolerance in maize seedlings. *Molecular Breeding, 39*(7), 97. https://doi.org/10.1007/s11032-019-1005-4

Zeng, X., Dai, Y.-J., Dickinson, R. E., & Shaikh, M. (1998). The role of root distribution for climate simulation over land. *Geophysical Research Letters, 25*(24), 4533–4536.

Zengler, K., Hofmockel, K., Baliga, N. S., Behie, S. W., Bernstein, H. C., Brown, J. B., Dinneny, J. R., Floge, S. A., Forry, S. P., Hess, M., Jackson, S. A., Jansson, C., Lindemann, S. R., Pett-Ridge, J., Maranas, C., Venturelli, O. S., Wallenstein, M. D., Shank, E. A., & Northen, T. R. (2019). EcoFABs: Advancing microbiome science through standardized fabricated ecosystems. *Nature Methods, 16*(7), 567–571.

Zhan, A., Schneider, H., & Lynch, J. P. (2015). Reduced lateral root branching density improves drought tolerance in maize. *Plant Physiology, 168*(4), 1603–1615.

Zobel, R. W., & Waisel, Y. (2010). A plant root system architectural taxonomy: A framework for root nomenclature. *Plant Biosystems, 144*(2), 507–512. https://doi.org/10.1080/11263501003764483

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