Bioenergy sorghum’s deep roots: A key to sustainable biomass production on annual cropland

Austin Lamb¹ | Brock Weers¹ | Brian McKinley¹ | William Rooney² | Cristine Morgan³ | Amy Marshall-Colon⁴ | John Mullet¹

¹Department of Biochemistry & Biophysics, Texas A&M University, College Station, Texas, USA
²Department of Soil & Crop Sciences, Texas A&M University, College Station, Texas, USA
³Soil Health Institute, Morrisville, North Carolina, USA
⁴Department of Plant Biology, University of Illinois, Champaign-Urbana, Illinois, USA

Correspondence
John Mullet, Department of Biochemistry & Biophysics, Texas A&M University, College Station, TX, USA.
Email: jmullet@tamu.edu

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Abstract
Bioenergy sorghum has high biomass yield potential, drought resilience, good nitrogen use efficiency, and a root system that contributes to the accumulation of soil organic carbon. In this study, field grown bioenergy sorghum root systems were analyzed during the growing season to characterize their depth, biomass, morphology, anatomy, and gene expression profiles. Bioenergy sorghum roots grew continuously during a 155-day growing season producing ~175 nodal roots, accumulating ~7 Mg of dry biomass per hectare, and reaching >2 m deep in the soil profile. Nodal roots within 20 cm of the stem were 1–5 mm in diameter, whereas roots deeper in soil profiles were enriched in lateral roots with small diameters (~30–500 µm) enabling growth through soil macropores. In older field-grown plants, roots with intact endodermal, vascular and inner root tissues were surrounded by degraded or aerenchyma-filled epidermal and cortical cell layers. Transcriptome analysis of nodal, surface, and deep roots identified >2,500 differentially expressed genes involved in root growth, transport, adaptation, defense, and AMF–root interaction. Deep roots (180–240 cm) differentially expressed genes that regulate lateral root growth. Surface roots (0–20 cm) located mid-row differentially expressed genes involved in nitrate transport, whereas ammonium transport genes were expressed in surface and deep roots and genes involved in phosphate transport were expressed in nodal, surface, and deep roots. Overall, bioenergy sorghum’s long growing season enables root systems to grow deeper and accumulate more biomass than annual grain crops such as maize, attributes that could help restore annual cropland soil organic carbon levels and improve soil productivity. Deep roots active in nutrient transport are positioned to take-up fertilizer leached deep into soil profiles mitigating potential nutrient run-off. Bioenergy sorghum’s large and deep root system is a key to sustainable production of biofuels, biopower, and bioproducts on annual cropland.

Keywords
bioenergy, nutrients, roots, sorghum, transcriptome
1 | INTRODUCTION

Atmospheric CO₂ levels have risen from ~270 ppm in pre-industrial times to 411 ppm in 2020, the highest level in ~800,000 years (Lindsey & Dlugokencky, 2019). At current rates of emission (~35 Gt/yr) CO₂ levels could reach 450–500 ppm by 2100 with potentially significant negative impacts on climate, coastal flooding, agricultural production, forest ecosystems, and wildlife habitat (Arnhet et al., 2019). The IPCC recently estimated that agriculture, forestry, and other land-use impacts from 2007 to 2016 accounted for ~23% of total net anthropogenic emissions of greenhouse gases (GHG) accounting for ~13%, ~44%, and ~82% of total CO₂, methane, and nitrous oxide emissions, respectively (Arnhet et al., 2019). Nitrous oxide emissions are primarily a consequence of widespread application of N-fertilizers to annual grain crops such as maize, in order to maximize grain yield. Unfortunately, excess N-fertilizer, not taken up by annual crop root systems, can leach from fields and contaminate ground water and waterways causing eutrophication (Hirel et al., 2007). Moreover, soil microbes convert a portion of the applied N-fertilizer to nitrous oxide, a potent GHG (Crutzen et al., 2008; Prather et al., 2012). Optimization of the rate and timing of N-fertilizer application, more efficient N-uptake by annual crop root systems, and enhanced secretion of biological nitrification inhibitors (Subbarao et al., 2017) are needed to reduce agricultural nitrous oxide emissions. Riparian strips (Meehan et al., 2013) and rotation of grain crops with deeper rooting bioenergy crops that efficiently take up N-fertilizer could help mitigate some of the negative environmental impacts of excess N-fertilization of annual grain crops.

Soils are the largest terrestrial carbon sink (~2400 PgC) (Batjes, 2000). Soil organic carbon (SOC) contributes to soil fertility and water-holding capacity increasing crop productivity (Davies, 2017; Hudson, 1994). Therefore, it is of great concern that ~133 Pg of carbon has been depleted from the top 2 m of soil profiles globally (Sanderman et al., 2018). Total root biomass, the depth and distribution of root systems, root biomass composition, and the soil-microbiome environment are important factors that modulate rates of SOC restoration (Kell, 2012). The depth of root systems in soil profiles depends on the duration of root growth and on the capacity of roots to penetrate soils with high impedance (Gao et al., 2016). Some root systems accomplish this by growing through ~30–300 µm pores in the soil matrix where they associate with microbes that help stabilize soil carbon (Kravchenko & Guber, 2017; Kravchenko et al., 2019). The root systems of perennial bioenergy grasses, such as switchgrass, can substantially increase the level of SOC (Martinez-Feria & Basso, 2020), demonstrating that plant-mediated restoration of SOC on land used for perennial crops could sequester a significant amount of atmospheric carbon dioxide (Anderson-Teixera et al., 2009). In the United States, annual cropland SOC levels have been depleted by 50% or more over the past 100 years (Sanderman et al., 2018). Restoration of annual cropland SOC would increase annual crop productivity and reduce the impact of drought on annual crop yield by increasing soil water-holding capacity (Davies, 2017; Hudson, 1994). The development and deployment of annual bioenergy sorghum hybrids that have large and deep root systems could help increase SOC and improve annual cropland productivity.

Bioenergy sorghum (Sorghum bicolor L. Moench) is a drought and heat resilient annual hybrid biomass crop that can be flexibly deployed in annual cropping systems for production of biofuels, biopower, and other bioproducts (Mullet et al., 2014; Rooney et al., 2007). The biomass yield of bioenergy sorghum ranges from ~10 to 48 Mg/ha depending principally on season length, the environment (i.e., water supply), and soil characteristics (Byrt et al., 2011; Gill et al., 2014; Moore et al., 2021; Olson et al., 2012; Truong et al., 2017). Although targeted for production on cropland marginal for annual food crops, bioenergy sorghum’s water use efficiency and seasonal biomass yield were higher than that of maize when grown in an optimal maize-growing region of the US Midwest (Moore et al., 2021). Conversion of biomass derived from bioenergy sorghum to biofuels or biopower provides ~75–90% GHG displacement (Olson et al., 2012) with a high Eo/Ei ratio (Byrt et al., 2011). The carbon intensity (C.I.) of ethanol production from bioenergy sorghum biomass was estimated to be ~17 gCO₂e/MJ (Kent et al., 2020), similar to corn stover, and significantly lower than corn grain ethanol (~52–105 gCO₂e/MJ) (Scully et al., 2021; US Environmental Protection Agency, 2009). However, the estimated C.I. value for bioenergy sorghum ethanol may have been high because the study assumed bioenergy crop production would have a negative impact on SOC although a 7-year study of an early generation bioenergy sorghum significantly increased SOC (Shahandeh et al., 2016).

Information on bioenergy sorghum’s root system is needed to understand why growing this type of biomass crop on annual cropland increased SOC (Shahandeh et al., 2016). Root system morphology, extent of proliferation in the soil profile, and transport functions and their impact on the uptake of water, N-fertilizer, and other nutrients from soil profiles need to be further characterized. There are also gaps in our understanding of the bioenergy sorghum root transcriptome and the sorghum root-microbiome. Therefore, in the current study, the root system of a field grown bioenergy sorghum hybrid was analyzed during the growing seasons of 2017–2020 to obtain information on root system development, biomass accumulation,
morphology, distribution in the soil profile, root anatomy, and transcriptome dynamics. This information may help improve the accuracy of C.I. estimates, root growth modeling, and fill gaps in our fundamental understanding of bioenergy sorghum root system function.

2 | MATERIALS AND METHODS

2.1 | Field plot management

Bioenergy sorghum hybrid, TX08001, was planted at the Texas A&M University Farm in Burleson County, TX on 5/2/17, 6/1/18, 6/4/19, and 6/5/20; plant emergence occurred 4–7 days after planting (Tables S1 and S2). Plot sizes were 16 rows by 100 m in 2017, 24 rows by 50 m in 2018, 44 rows by 30 m in 2019, and 32 rows by 30 m in 2020. At planting, a solution of liquid ammonium polyphosphate (11–37–0), UAN 32%, and zinc sulfate was applied to the same planting depth 5 cm to the side of the seed to yield 45–63−0 +5 Zn (kg/ha). In 2017 and 2018, the plots were side dressed with 123 kg/ha N (UAN 32%) at 4 weeks after emergence at 21 days after emergence (DAE) to 15 cm between plants. In 2017 and 2019, a sub-plot was also thinned to 15 cm starting at 32 days after emergence (DAE) and while the soil in the sand site consists of a Weswood Silt Loam (Soil-Survey-Staff, 2020), while the soil in the sand site was measured during the growing season. Stalks were cut immediately above the uppermost ring of emerged nodal roots. Leaf sheaths and leaves were removed, separated, weighed, dried at 71°C for 3 days, and then re-weighed. Stem length and stem fresh and dry weights were also measured. Stem measurements included the lower portion of stems after removal of roots. Root systems were excavated from a volume of soil 30 cm deep, 30 cm wide centered on the culm, and 90 cm along the row with a hand spade. This 90 cm length of excavation contained the roots of five plants per harvest. The extracted roots were immersed in water for 1 h to loosen soil before rinsing off any remaining soil. Cleaned roots were stored at 4°C in sealed plastic bags for analysis. Root systems were cut in half and residual soil was washed off prior to imaging. Root system halves were positioned on an Epson Expression 10000 XL scanner (Seiko Epson Corporation) in a custom 50 x 80 cm glass tray with 2.5 cm tall aluminum walls containing water to a depth of 2 cm. Images were acquired at 600 DPI. The trays are longer and wider than standard trays so that the tray edges are not included in scans. The scanner was outfitted with 3 cm long ½” Schedule 40 PVC pipe to accommodate the deeper tray size. All root scan analysis was conducted using WinRHIZO software (Regent Instruments) with default parameters. Resulting images were compiled and batch processed using the maximum detection option. The number of nodal roots and nodal root rings initiated above ground were counted and nodal root fresh and dry weights were determined. Root dry weight per hectare was calculated from the mean sum of all roots (both above and below ground) per plant. SigmaPlot v.11 (Systat Software, Inc.) was used to perform the analysis. Root system analyses were conducted using five replicates. The 2017 root morphology experiment analyzed stems, leaves, leaf sheaths, and roots harvested at 32, 64, 96, and 155 DAE. At 155 DAE, five root-containing soil cores 10 cm in diameter were obtained to a depth of 1.2 m at the culm, 19 cm from the culm, and 38 cm from the culm using a Giddings Machine Company #25-SCS Hydraulic Soil Sampling, Coring, and Drilling Machine (probe truck) in the sub-plot thinned to 100 cm for a total of 15 cores. Cores were sectioned into 25 cm segments before roots were recovered. Harvested stems were cut at the soil level and placed in paper bags for drying. Root samples in 2018 were taken in row, 19 cm from the row to a depth of 1.2 m, and at 38 cm from the row to a depth of 2.4 m at 120–140 DAE. In 2018, 10 soil cores were obtained from plots thinned to 15 cm plant spacing in row, from both soil types, using the probe truck. In row and 19 cm from the row, soil cores were obtained to a depth of 1.2 m, and while samples 38 cm from the row were taken to a depth of 2.4 m (from 1.2 to 2.4 m, there are only five replicates per site) totaling 35 cores per site. Samples from the sand site were collected at 120 DAE while samples from the clay site were harvested at 140 DAE due to inclement weather conditions. Soil cores were sectioned into 20 cm increments, placed in 1 G bags filled halfway with water, allowed to sit overnight at 40°C, washed on a 1 mm² mesh, and roots were collected for storage at 40°C before being scanned in the same manner as root samples from 2017. In 2019, five plots thinned to 15 cm in row were harvested using the trenching technique at 60 DAE from the sand plot using the same techniques from 2017.
2.3 Transcriptome analysis

Root tissue samples for RNA-Seq analysis were harvested from TX08001 plants growing in the field in Weswood Silt Loam at 120 DAE using the soil probe truck, and the probe from 2017, to a depth of 2.4 m. Soil cores were sectioned into 20 cm segments before being washed in a water filled 150 G Rubbermaid Structural Foam Stock Tank on a 1 mm² mesh screening box used to wash soil from roots. Roots were collected from the screen using forceps, placed in Whirl-Pak sample bags, and flash-frozen in liquid nitrogen. Nodal roots that were embedded in soil but lacking lateral roots were identified and a 6 cm section of nodal root tissue that included the root tip was harvested for analysis. Roots present at mid-row (38 cm from stems) located in the top 20 cm of the soil profile (surface roots, SR) or 180–240 cm deep in the soil profile (deep roots, DR) were also collected for transcriptome analysis. Tissues were ground into a fine powder with a heat sterilized mortar and pestle filled with liquid nitrogen. The ground tissue was transferred into liquid-nitrogen chilled sterile 1.5 ml centrifuge tubes. RNA was extracted using the Zymo RNA mini-prep kit Purity and concentration of the RNA was analyzed using a Thermo ScientificNanoDrop One Microvolume UV-Vis Spectrophotometer before being sent for fragmentation analysis on a 5300 Fragment Analyzer using software version 3.1.0.12. RNA that passed QC was sent to the Joint Genome Institute for sequencing to a depth of 30–50 million reads. Sequenced reads were aligned to the *Sorghum bicolor* V3.1 genome using HISAT2 aligner (Daehwan et al., 2015; McCormick et al., 2018). The transcriptome assembly and TPM normalization were conducted using StringTie version 1.3 (Pertea et al., 2015) and relative gene expression used TPM normalized data. The script prepDE.py was used to convert nucleotide coverage data from StringTie into read counts that were readable by differential expression statistical packages. For differential gene expression analysis, the nodal root (NR) and SR tissues were represented by three replicates and DR tissue by four replicates. Functional annotations of the transcripts were obtained from the *Sorghum bicolor* V3.1 genome which is available from Phytozone 12 (McCormick et al., 2018). Visualization of the relationships between biological replicates of NR, SR, and DR was performed with the use of multidimensional scaling using the plotMDS function available in the EdgeR package (Robinson et al., 2010). Read counts were cpm normalized as input to construct an MDS plot (Robinson et al., 2010). The DESeq statistical package was used to identify genes that were differentially expressed in comparisons of NR and SR, NR and DR (Anders & Huber, 2010). Transcripts that were differentially expressed in the NR-SR comparison were split into two categories, NR and SR, with transcripts that were upregulated in NR placed in the NR category and transcripts that were upregulated in SR relative to NR placed in the SR category. This was repeated with the NR-DR, and the SR-DR comparisons to create a total of three groups: NR, SR, and DR. Transcripts that the SR and DR categories had in common were moved to a fourth category called SRDR. Tau for tissue specificity was calculated as described in Yanai et al. (2005). The TPM normalized data set from TX08001 root samples (NR, SR, and DR) was used for Tau analysis of differential expression among root samples. TPM normalized data from a panel of leaf, stem, and panicle tissues from BTx623 and target root tissues from TX08001 was also used for Tau analysis. Both data sets included genes with expression ≥4 TPM in at least one tissue included in the analysis. Transcripts that were differentially expressed (10-fold, 4 TPM) among NR, DR, and SR were subjected to gene ontology (GO) enrichment analysis conducted using plant reg map (Tian et al., 2020). A p-value threshold of ≤0.005 was used to identify GO terms of significance.

2.4 Microscopy

Root samples of 38 cm, used for microscopic analysis, were collected in 2020 from the row to a depth of 2.4 m at ~100 DAE using a 10 cm diameter core with the probe truck. Cores were processed in the field in the same way as roots for RNA extraction. Washed roots were collected into 20 ml scintillation vials filled with 18 ml of Trump fixative (McDowell & Trump, 1976). The tissues were left in fixative at 4°C on a shake table for 4 days. The tissues went through a dehydration series into ethanol in 10% increments then transferred into isopropanol through a five-step series in 20% increments and transferred into xylene through a five-step series in 20% increments. Tissues were transferred into Paraplast X-tra in a three-step series in 33% increments and exchanged with pure Paraplast X-tra twice more. Paraplast X-tra steps were conducted in an oven set at 70°C and allowed to incubate for 24 h between fluid changes. Tissues were embedded in Paraplast X-tra and sectioned at 12 µm thickness. Sections were mounted to chrome-alum stubbed slides. Mounted slides were placed on a slide warmer to flatten the sections for 10 minutes. Slides were transferred to an oven set at 40°C overnight to dry the slides. Slides were deparaffinized in xylene (10 min), rehydrated in a five-step ethanol series (5 min, 20% increments), placed in pure water (5 min), and stained in FASGA (10 min) (Tolivia & Tolivia, 1987). After staining, slides were de-stained in water (1 min), dehydrated in another five-step ethanol series (30 s, 20% increments), and placed back in xylene (1 min) before
using 70 µl of Permount to adhere a xylene soaked #1 ½-24 x 50 mm coverslip and placed in an oven at 40°C for 2 days to fully cure the Permount. Slides were visualized on a Leica DM6B light microscope. Micrographs were taken using a Leica DMC 4500 5-megapixel color camera at a magnification between 5 and 40× and processed using LAS X software from Leica.

3 | RESULTS

3.1 | Experimental plan

The results described in this study were obtained from field experiments conducted from 2017 to 2020 (Tables S1 and S2). Basic characterization of bioenergy sorghum hybrid root system development and plant biomass accumulation during a 155-day growing season was collected in 2017 (Table S1: 2017a; Figure 1). A baseline of information about the distribution of the bioenergy sorghum root system in the soil profile and root morphometrics was also collected in 2017 (Table S1: 2017b; Figure 2). Root tissue for transcriptome analysis was obtained from nodal roots and surface (0–20 cm) and deep (180–240 cm) roots mid-row from the 2018 field plots (Table S1: 2018a; Tables 1–3). In 2018, bioenergy sorghum was grown in plots rich in sand or clay and data on root system depth and morphometrics was collected (Figure S1: 2018b; Figure 4). In 2019, additional information on the morphometrics of bioenergy sorghum roots of plants grown in the sand plot was investigated at 64 DAE (Table S1: 2019; Figure 3). In 2020, roots of bioenergy sorghum grown in the sand plot were collected for microscopic analysis of root anatomy (Figure S1: 2020; Figure 5).

3.1.1 | Bioenergy sorghum root biomass increases throughout the growing season

Bioenergy sorghum hybrid root system development was monitored by excavating roots contained in a volume of soil centered on rows every 32 days during a 155-day growing season in 2017 (Tables S1 and S2). Stem and leaf plus leaf sheath dry weight was recorded, and root biomass was measured after removing soil from root systems (Figure 1). Total plant dry biomass accumulated steadily from 32 days after plant emergence (DAE) until harvest reaching ~550 g/plant (Figure 1a), consistent with Olson et al. (2012). At harvest, root biomass in the excavated portion of the soil profile was 80 g/plant (~6.7 Mg/ha), 14% of total plant biomass. The accumulation of root biomass during the season was correlated with an increase in nodal root number from ~30 at 32 DAE to ~175 at the end of the growing season (Figure 1b). Nodal root production slowed during the last month of the season possibly due to cooler weather in the fall. Aerial nodal root tips were covered with a thick mucilage from emergence until roots were embedded in the soil profile where this trait was no longer monitored. The last 25 nodal roots that grew out
Figure 2 Root system distribution and morphometrics of field grown bioenergy sorghum. Bioenergy sorghum root system distribution, biomass, and morphometric data collected at the end of the 2017 growing season: (a) Root system metrics as a function of depth in the soil profile (25 cm intervals to a depth of 120 cm) in row: Root diameter (top panel), root dry weight (mg/soil volume (cm$^3$)) (middle panel), and root length density (cm/cm$^3$ of soil volume) (lower panel). (b) Root system metrics of roots in 120 cm columns of soil profiles as a function of distance from the row: Average root diameter in row (0 cm), 19 cm from the row, and mid-row (38 cm) (left panel); root dry weight (g) 0, 19, and 38 cm from the row (middle panel) and root length density (cm/cm$^3$), 0, 19, and 38 cm from the row (right panel)
from above ground stem nodes and reached a length of 4–6 cm without reaching the soil profile.

### 3.1.2 Root system distribution in soil profiles

The extent of root proliferation of individual bioenergy sorghum plants in soil profiles was assessed in 2017 by thinning TX08001 to a spacing of 1 m to reduce intermixing of root systems from adjacent plants. Tills were removed to maintain plant density and prevent tiller root system development. At the end of the growing season (155 DAE), soil cores 120 cm deep were collected directly below individual plants and between rows at a distance of 19 cm and 38 cm (mid-row) from plant stems. Soil cores were divided into 25 cm sections of increasing soil depth and roots in each volume of soil were collected, imaged, and fresh and dry weights measured (Figure 2). Roots in the top 25 cm of the soil profile and lower in deeper portions of the soil profile (Figure 2a, lower panels). Roots in 120 cm soil columns directly under plants in rows had average diameters of 0.73 mm, whereas roots in 120 cm soil columns 19 cm from the stem and at mid-row (38 cm) had average diameters of ~0.3 mm similar to roots more than 25 cm deep in the soil profile in rows (Figure 2b). Root length density (cm/cm³) below stems (25–120 cm deep) and 19 cm from the stem (0–120 cm deep) were similar, whereas 38 cm from the stem (0–120 cm deep), root length density was about 15–20% lower (Figure 2b). Taken together, the root system of the bioenergy sorghum TX08001 is composed of nodal roots that are >1 mm in diameter until they extend more than ~20 cm from the stem and small diameter roots averaging ~0.3 mm, that occupy the rest of the soil profile.

### 3.1.3 Bioenergy sorghum root system morphology

Root systems associated with 64-day-old field grown plants in 2019 were excavated to a depth of 30 cm, washed, scanned, and analyzed using WinRHIZO to characterize the morphology of this portion of the root system in terms of diameter, length, surface area, and volume (Figure 3).
FIGURE 4  Bioenergy sorghum root dispersion by depth and soil type. Roots were collected by probe truck to a soil depth of 240 cm from field-grown plants 120 DAE (sand) or 140 DAE (clay) in 2018. (a) TX08001 root length density (cm/cm³), (b) average root diameter (mm), and (c) root tissue density (g/cm³ of root volume) versus soil depth (m) taken at mid-row of TX08001 grown in sand (dark gray) or clay (light gray). Replicate information may be found in Table S3.
The analysis showed that ~27% of total root length was accounted for by roots with diameters >1 mm and ~55% by roots with diameters <0.5 mm (Figure 3a). The diameter of nodal roots decreases gradually as a function of distance from the stem and more rapidly once nodal roots penetrate the soil profile. On average, the diameters of nodal roots more than 18–20 cm from the stem in the soil profile were <1 mm. Nodal roots with large diameters (>1 mm) have relatively high surface area and volume to length ratios compared to small diameter nodal roots (<1 mm) that have ~30-fold greater length per unit root volume (Figure 3b). Branching from nodal roots >1 mm in diameter was infrequent, whereas numerous lateral roots of several orders were associated with nodal roots <1 mm in diameter collectively generating ~15,500 root tips per plant in the portion of the root system analyzed after 60 days of plant development.

### 3.1.4 Roots proliferate >2 m deep in sandy and clay-rich soils

Bioenergy sorghum root proliferation in different soil types was investigated in 2018 and 2019 by planting TX08001 in plots with sandy or clay-rich soils using standard row and plant spacing. The sand and clay content of the soils at the two sites was significantly different at all depths analyzed (Figure S1). The distribution of bioenergy sorghum root systems in the two soil types was analyzed to a depth of 3 m late in the growing season (120–140 DAE). In the sand plot, roots were detected to a depth of ~2.4 m, while in the clay plot, roots reached a depth of ~2 m. At mid-row, RLD decreased gradually from the top of the soil profile to these depths (Figure 4a). The average diameter of roots was ~0.25–0.3 mm at soil depths below ~20 cm (Figure 4b). The specific root density (biomass/root volume) of large diameter nodal...
roots (>1mm) located in the upper portion of the soil profile within 20 cm of the stem was 2–4-fold greater than small diameter roots (~0.25–3 mm) located more than 20 cm from the stem in the soil profile or at mid-row (~0.21 g/cm³) (Figure 4c; Table S3). Therefore, small diameter roots that have relatively low specific root density require at least 50-fold less investment of biomass per unit root length compared to large diameter nodal roots. The small diameter roots predominate in >90% of the soil volume explored by bioenergy root systems, whereas large diameter nodal roots (>1 mm) are present only in close proximity (within 20 cm) to plant stems. Data on root length density and specific root density of small diameter roots that occupy >90% of the soil volume were used to estimate that this portion of the root system contributes ~0.3 Mg/ha to the overall root biomass of bioenergy sorghum. The biomass of the bioenergy sorghum root system within 30 cm of the stem that includes nodal roots was ~6.7 Mg/ha; therefore in total, the bioenergy sorghum root system biomass was ~7 Mg/ha at the end of the growing season.

3.1.5 | Bioenergy sorghum root anatomy

Cross-sections of nodal roots and lateral roots were collected from field grown plants in 2020 to characterize the diameters and anatomy of bioenergy sorghum roots (Figure 5). Cross-sections of large diameter nodal roots revealed typical C4 grass nodal root anatomy including the epidermis, cortical cells, endodermis, numerous large metaxylem, phloem, and parenchyma cells located in the center of the root (Figure 5a). The cross-sections of nodal roots of older plants often showed the presence of aerenchyma throughout the outer cortical cell layers indicating turnover was occurring (Figure 5b). Nodal roots of field-grown plants embedded in soil obtained later in the season sometimes retained only remnants of the epidermal and cortical cell layers attached to an inner root core comprised of vascular tissues surrounded by a lignified endodermis (Figure 5c). Lateral roots had diameters ranging from ~30 to 500 µm (Figure 5d). These roots also had an outer epidermis, cortical cell layer, endodermis, and in most cases, a large metaxylem channel at the center of each lateral root. Some lateral roots from field-grown plants showed conversion of cortical cells to aerenchyma and occasionally, the presence of blue-staining structures that may correspond to AMF (Figure 5e). In many cases, the epidermis and cortical cells of lateral roots were degraded leaving an intact but lignified endodermis and interior vascular tissues (Figure 5f). The degradation of root cortical and epidermal cell layers may indicate that bioenergy sorghum remobilizes carbon and nitrogen from these tissues while retaining root transport functions.

3.2 | Transcriptome analysis of bioenergy sorghum roots

Transcriptome analysis was carried out to obtain information about the functional specialization of bioenergy sorghum roots and their adaptation to surface and deep soil environments. In 2018, tissue for RNA-seq profiling was obtained from nodal roots (NR) that had become embedded in the soil but prior to lateral root proliferation and root systems enriched in lateral roots located 38 cm from the stem (mid-row) that were in the top 20 cm of the soil profile (surface roots, SR) or 180–240 cm deep in the soil profile (deep roots, DR). Comparison of RNA-seq data from NR, SR, or DR root systems identified 1696 transcripts that were differentially expressed 10-fold or greater in NR, 498 in SR, and 363 in DR (FDR <0.05, TPM >3) (Figure 6; Tables S4–S7). In addition, there were 1785 genes differentially expressed 10-fold or greater in both SR and DR (SRDR) compared to NR. The general functions of the differentially expressed root genes were analyzed using GO enrichment (Figure 6; Tables S8–S11). Differentially expressed genes (DEGs) in NR were enriched in 187 GO categories including metabolism, structure, regulation, and photosynthesis. DEGs identified in SR were enriched in GO categories related to chelation, degradation, and metabolism (Figure 6). DEGs in SRDR were enriched in GO categories related to degradation, transport, and defense (Figure 6).

3.3 | Tau analysis of differential expression in roots

Tau analysis is a useful method for identifying genes that are differentially expressed in target tissues of interest relative to several other tissues or “states” (Yanai et al., 2005). Tau analysis of gene expression in NR, SR, and DRs identified 1191 genes in NR, 237 genes in SR, and 287 genes in DR with Tau values 0.9 or higher. Tau analysis was subsequently used to investigate whether the genes differentially expressed in NR, SR, and DR were also differentially expressed in roots relative to shoot tissues. To do this, the genes with >0.9 Tau values identified in comparisons of NR, SR, and DR were further analyzed by comparing their expression in roots to leaves, leaf sheaths, stems, panicles, and seeds obtained from BTx623 (McCormick et al., 2018). This analysis showed that 25% (NR), 90% (SR), and 60% (DR) of these genes had Tau values of 0.9 or greater when compared to the panel of BTx623 tissues. The results indicate that differentially expressed genes in NR were more similar to the panel of above ground tissues compared to SR and DR. This is consistent with differential expression of a large number of genes involved in photosynthesis in NR.
Among the NR genes with high Tau values in both root and shoot analyses was a homolog of \textit{AtCCD8}/MAX8 (Sobic.007G170300), a gene that encodes a key enzyme involved in strigolactone biosynthesis, a plant hormone that regulates tillering and early events in AMF association with roots (Gutjahr & Parniske, 2013). A more detailed analysis of genes with high Tau values that are expressed in DR and SR is provided below. An in-depth analysis of genes expressed in nodal roots will be the focus of subsequent studies.

### 3.3.1 Genes differentially expressed in deep roots

Genes with high Tau values in DR identified in comparisons of NR, SR, and DR were sorted into functional categories using Mapman and information about the function of Arabidopsis homologs used to examine potential roles of the corresponding sorghum genes (Table 1). For example, a sorghum homolog of \textit{WOX11} (Sobic.002G421800), a WUSCHEL-homeobox transcription factor involved in the formation of lateral roots (Baesso et al., 2018), was differentially expressed in deep roots consistent with extensive proliferation of lateral roots deep in the soil profile. A sorghum homolog of \textit{ATC} (Sobic.006G068300) was differentially expressed in DR. ATC encodes a mobile TFL-like protein that can interact with FD and inhibit floral initiation (Huang et al., 2012). In sorghum deep roots, \textit{SbATC} could be involved in long-distance signaling to the shoot or regulation of root meristem interaction with FT-like peptides that modulate growth. The sorghum homolog of \textit{ATAM} (Sobic.003G103701) that is differentially expressed in DR could be involved in root stem cell niche and meristem maintenance as occurs in Arabidopsis (Li et al., 2021). Sorghum homologs of \textit{bZIP29} (Sobic.007G182500), a regulator of root cell number (Van Leene et al., 2016; Wang et al., 2020), and \textit{ADA2B} (Sobic.001G088100), a regulator of trichome density (Kotak et al., 2018) and growth (Rao & Virupapuram, 2021), could regulate root hair density or growth of deep roots. The sorghum homolog of \textit{AtPLAIVA} (Sobic.005G159400), a regulator of lateral root development in response to phosphate deficiency (Rietz et al., 2010), may serve a similar function in DR. Microscopic analysis of DR showed the presence of aerenchyma; therefore, differential expression of a homolog of \textit{bZIP21} (TGA9), a gene involved in autophagy (Wang et al., 2020) and ROS-pathogen signaling (Noshi et al., 2016), indicates that the sorghum homolog may be involved in root turnover/pathogen defense in DR.

Grass root growth and lateral root formation are regulated by auxin (IAA) (Yu et al., 2016). Therefore, differential expression in DR of a sorghum homolog of \textit{YUCCA3} (IAA biosynthesis) (Sobic.002G1230300) and the SAUR-like auxin responsive protein \textit{PAPI} (IAA26) (Sobic.003G035700) that forms a negative feedback loop with HECATE proteins in Arabidopsis (Zhu et al., 2016) is consistent with IAA regulation of DR growth. ABA...
**TABLE 1**  Genes differentially expressed in deep roots of field-grown bioenergy sorghum. Sorghum genes expressed in roots 180–240 cm deep in soil profiles were compared to nodal and surface roots to identify genes with high Tau values (>0.9, highlighted in blue). The genes were sorted into predicted functional categories (i.e., development, hormones, signaling) based on identification of the closest *A. thaliana* homolog (best hit, gene name, function). Gene expression is reported in TPM.

| Transcript ID       | Gene name | Function                          | Nodal roots | Surface roots | Deep roots | Tau  |
|---------------------|-----------|-----------------------------------|-------------|---------------|------------|------|
| **Development/Transcription factors** |            |                                   |             |               |            |      |
| Sobic.002G421800.2  | AT3G03660.2| WOX11 WUSCHEL related homeobox 11 | 0           | 6             | 29         | 0.90 |
| Sobic.006G068300.1  | AT2G27550.1| ATC, TFL1-like                     | 0           | 0             | 36         | 1.00 |
| Sobic.003G103701.7  | AT3G48190.1| ATM Ataxia-telangiectasia mutated | 2           | 1             | 13         | 0.91 |
| Sobic.007G182500.3  | AT4G38900.3| bZIP29-like                        | 0           | 0             | 12         | 1.00 |
| Sobic.001G088100.2  | AT4G16420.1| ADA2B ADA2B                        | 0           | 12            | 59         | 0.90 |
| Sobic.005G159400.1  | AT4G37070.2| PLAI3A  Acyl transferase           | 0           | 0             | 6          | 0.97 |
| Sobic.009G155050.8  | AT1G08320.1| bZIP21 bZIP transcription factor   | 0           | 11            | 69         | 0.92 |
| **Plant hormones**  |            |                                   |             |               |            |      |
| Sobic.002G120300.2  | AT1G04610.1| YUC3 YUCCA 3                      | 1           | 2             | 15         | 0.92 |
| Sobic.003G035700.2  | AT3G16500.1| IAA26.PAP1 Phytochrome-associated protein 1 | 3 | 9             | 67         | 0.92 |
| Sobic.001G424400.1  | AT1G07430.1| HAI2 Highly ABA-induced PP2C gene 2 | 0           | 2             | 9          | 0.90 |
| Sobic.006G018400.1  | AT5G1760.1 | AHG1 Protein phosphatase 2C        | 0           | 1             | 7          | 0.92 |
| Sobic.003G313800.2  | AT1G75450.1| CKX5 Cytokinin oxidase 5           | 0           | 1             | 9          | 0.91 |
| **Signaling**       |            |                                   |             |               |            |      |
| Sobic.002G208800.3  | AT2G29110.1| GLR2.8 Glutamate receptor 2.8      | 0           | 1             | 6          | 0.95 |
| Sobic.007G135300.1  | AT3G57230.1| AGL16 AGAMOUS-like 16             | 0           | 10            | 145        | 0.97 |
| Sobic.006G261300.5  | AT2G37250.1| ADK Adenosine kinase               | 0           | 0             | 5          | 1.00 |
| Sobic.008G029400.1  | AT2G46400.1| WRKY46 WRKY DNA-binding protein 46 | 2           | 1             | 17         | 0.91 |
| Sobic.005G069800.1  | AT1G12720.1| WAK2 Wall-associated kinase 2      | 0           | 6             | 38         | 0.92 |
| Sobic.007G216000.1  | AT1G21240.1| WAK3 Wall-associated kinase 3      | 0           | 2             | 8          | 0.90 |
| Sobic.003G402100.2  | AT5G07280.1| EMS1.EXS Leucine-rich repeat kinase| 0           | 0             | 5          | 1.00 |
| Sobic.001G044700.2  | AT2G19830.1| SNF7 SNF7                         | 0           | 0             | 30         | 1.00 |
| **Plant defense**   |            |                                   |             |               |            |      |
| Sobic.010G124800.1  | AT2G37710.1| RLK Receptor lectin kinase         | 0           | 0             | 4          | 0.93 |
| Sobic.002G109500.1  | AT5G48485.1| DIR1 Bifunctional inhibitor        | 0           | 33            | 182        | 0.91 |
| Sobic.004G022200.1  | AT3G46860.1| PR6 Serine protease inhibitor      | 0           | 23            | 172        | 0.93 |
| Sobic.010G247600.1  | AT4G31750.1| WIN2 HOPW1-1-interacting 2        | 0           | 5             | 42         | 0.94 |
| Sobic.009G174300.1  | AT3G56400.1| WRKY70 WRKY DNA-binding protein 70 | 0           | 1             | 13         | 0.97 |
| **Transport**       |            |                                   |             |               |            |      |
| Sobic.008G094300.1  | AT5G23660.1| SWEET12 MtN3                      | 0           | 2             | 13         | 0.91 |
| Sobic.001G373600.1  | AT3G48740.1| SWEET11 Nodulin MtN3              | 0           | 6             | 49         | 0.94 |
| Sobic.001G362500.1  | AT5G18840.1| Major facilitator superfamily protein | 0           | 4             | 39         | 0.94 |
| Sobic.001G323600.1  | AT3G18330.1| PMT5 Sugar transporter 5          | 0           | 1             | 6          | 0.90 |
| Sobic.009G142800.5  | AT5G09220.1| AAP2 Amino acid permease 2        | 1           | 7             | 53         | 0.92 |
| Sobic.006G077500.1  | AT1G25270.1| aa export seed Nodulin MtN21      | 0           | 0             | 6          | 0.96 |
| Sobic.003G267100.1  | AT2G47000.1| ABCB4 ATP-binding cassette subfamily B4 | 4           | 63            | 355        | 0.91 |
| Sobic.001G183700.1  | AT4G13420.1| HAK5 High affinity K+ transporter 5 | 2           | 20            | 110        | 0.90 |
| Sobic.007G120200.2  | AT3G47790.1| ATH7 ABC2 homolog 7              | 0           | 0             | 54         | 1.00 |
| Sobic.003G133100.2  | AT3G43790.1| ZIFL2 Zinc-induced facilitator-like 2 | 0           | 4             | 36         | 0.95 |
plays a key role in mediating plant responses to water deficit and this hormone helps maintain root growth under conditions of water limitation (Creelman et al., 1990; Sharp et al., 2004). As such, it was interesting to find a sorghum homolog of HIA2 (Sobic.001G424400) and AHG1 (Sobic.006G018400), genes that encode PP2C proteins that are involved in ABA signaling (Nishimura et al., 2007; Umezawa et al., 2009), among the genes that are differentially expressed in deep roots. A sorghum homolog of a gene involved in cytokinin metabolism, ATCKX5 – a cytokinin oxidase (Sobic.003G313800), was also differentially expressed in deep roots.

Numerous sorghum homologs of genes involved in signaling were identified including a homolog of ATGLR2 (Sobic.002G208800), a glutamate receptor. Sorghum homologs of genes encoding wall-associated kinases (WAK2, WAK3) (Kohorn et al., 2009) were identified indicating cell wall (pectin)-mediated signaling may regulate deep root function. A sorghum homolog of ATWRKY46 (Sobic.008G029400), a gene that regulates lateral root growth in response to osmotic stress/ABA signaling (Ding et al., 2015), was differentially expressed in DR. WRKY46/54/70 are important signaling components involved in brassinosteroid-regulated growth and responses to drought (Chen et al., 2017). Differential expression of a sorghum homolog of adenosine kinase (Sobic.006G261300), which interacts with SnRK1 (Mohannath et al., 2014), indicates regulation of energy homeostasis is important in deep roots. In addition, a large number of the genes differentially expressed in deep roots play roles in plant defense (Table 1). Several sorghum homologs of genes that are involved in defense were identified including an SA-signaling RLK (Sobic.010G124800), a homolog of DIR1 (Sobic.002G109500) (Isaacs et al., 2016), a serine protease inhibitor (PR6) (Sobic.004G022200) (Sels et al., 2008), WIN2 (Sobic.010G24750) (Lee et al., 2008), and WRKY70 (Sobic.009G174300) (Li et al., 2017). A number of sorghum homologs of genes that encode transporters were also differentially expressed in deep roots (Table 1). Among this group of genes were homologs of SWEET11/12 sucrose transporters (Sobic.008G094300, Sobic.001G373600) that may modulate the priming of SA-mediated defense responses (Gebauer et al., 2017), sugar and polyol transporters (Sobic.001G323600) (Klepke et al., 2005), an amino acid permease AAP2 that mediates xylem–phloem transport (Sobic.009G142800) (Zhang et al., 2010), and a transporter involved in amino acid export from seeds (Sobic.006G07750). Several other transporters were identified with potential functions in auxin transport (Sobic.003G267100) (Cho et al., 2012), potassium (Sobic.001G183700), and zinc transport (Sobic.003G133100).

3.3.2 | Genes differentially expressed in surface roots

Genes differentially expressed in surface roots (Tau 0.9 or greater) (Table 2) were sorted by Mapman and their potential functions analyzed as described above. Numerous sorghum genes involved in signaling were differentially expressed in SR including homologs of HAT14 (Sobic.004G185200), a gene involved in transcriptional regulation of nitrogen metabolism (Gaudinier et al., 2018), RAP2.11 (Sobic.002G350500), a gene that mediates responses to low potassium (Kim et al., 2012), NF-YB3 (Sobic.007G059500) a regulator of heat stress responses (Sato et al., 2014), and NF-NYC2 transcription factor involved in nutrient sensing (Brumbarova & Ivanov, 2019). In addition, a homolog of AHB1 (hemoglobin1) (Sobic.001G449600) a gene involved in NO and ABA-signaling (Rubio et al., 2019), PLD1 (Sobic.008G183400) a source of lipid signals that regulate growth and defense (Zhao, 2015), and GL22 (Sobic.002G135500) one of three sorghum genes encoding germin-like proteins that regulate root growth (Ham et al., 2012). A number of genes involved in plant defense were identified including a homolog of GP4 (Sobic.002G135700), four gene homologs of OSM34 (Sobic.008G182800), homologs of PR1 (Sobic.002G023300), six PR6 genes (Sobic.004G021700), and a gene encoding chitinase (Sobic.003G363900). GO enrichment indicated that genes involved in nitrogen metabolism were over-represented in SR. Consistent with this finding, sorghum homologs of nitrate reductase (Sobic.004G312500), nitrite reductase (Sobic.008182800), and 5 genes involved in nitrate transport (i.e., Sobic.004G202400, Sobic.004G009500, Sobic.004G09400, Sobic.003G270800, Sobic.003G188200) were differentially expressed in SR. Two phosphate transporters (Sobic.006G26800 and Sobic.001G502000) were also differentially expressed in surface roots. A sorghum gene homolog of ATNLM2 (NIP1;2) (Sobic.010G164100), a gene involved in aluminum transport, two genes involved in boron transport (Sobic.007G039600, 003G47900), and a gene that mediates iron transport (Sobic.006G152900) were also differentially expressed in surface roots.

3.3.3 | Differentially expressed genes encoding transporters

Genes involved in nutrient transport in SR and DR were targeted for further analysis because efficient uptake of nitrogen, phosphate, and other nutrients by bioenergy sorghum root systems from the soil profile contributes to sustainability and resilience. The sorghum genome encodes 1233 genes (1367 transcripts) annotated as transporters (Phytozome). Tau analysis was used to investigate the
expression of sorghum genes annotated as encoding transporters of nitrate, ammonium, or phosphate (Table 3). Two of the thirteen sorghum genes annotated as encoding nitrate transporters were highly differentially expressed in NR, five genes with Tau values >0.9 were expressed in SR, three genes were differentially expressed in SR and DR relative to NR, and two genes were expressed in all three root system types. In contrast, none of the seven ammonium transporters were differentially expressed in NR, one gene was differentially expressed in SR, four were more highly expressed in SR and DR compared to NR, and one was expressed in all roots. In all, 23 sorghum genes were annotated as encoding phosphate transporters. Four of these genes were differentially expressed in NR including Sobic.004G19900 with a Tau value of 0.96. Three genes were differentially expressed in SR including two genes (Sobic.006G026800 and Sobic.001G502000) with Tau values of 0.9 and 0.95, three genes in SR and DR, while 13 genes were expressed in all three root systems with relatively low Tau values (Table 3). Taken together, the analysis showed that several genes involved in nitrate transport are highly differentially expressed in SR, numerous ammonium transporters are expressed both in SR and DR, and the large number of phosphate transporters were differentially expressed in NR, SR, and DR.
SR, and DR, or expressed in all of the root systems analyzed. This information provides a starting point for determining the subcellular location and regulation of genes encoding nutrient transporters that are differentially expression bio-energy sorghum root systems.

### 3.3.4 Genes induced in roots by AMF

The association of arbuscular mycorrhizae fungi (AMF) with plant roots plays an important role in nutrient uptake by crops helping to reduce the loss of soil nutrients through leaching (Cavagnaro et al., 2015; Harrison, 2012). Interaction of AMF with sorghum roots induces the expression of a large number genes including a number of transporters (Watts-Williams & Cavagnaro, 2018). Therefore, we investigated whether the previously identified AMF-inducible root genes were differentially expressed in NR, SR, or DR in our study. The analysis showed that 98% of the previously identified mycorrhiza-inducible genes in sorghum root were highly differentially expressed in SR and DR relative to NR (10-fold DEG cutoff, p-value ≤0.05, FDR ≤0.05, and an expression threshold of TPM ≥4) (Watts-Williams et al., 2019). This result is consistent with prior studies showing that AMF have minimal association with large diameter roots compared to small diameter lateral roots in rice (Fiorilli et al., 2015). Tau analysis of AMF-induced root genes in SR and DR relative to shoot tissues. Genes encoding phosphate transporters (Sobic.003G243400 and Sobic.006G026800) and an ammonium transporter (Sobic.003G370400) were among this set of AMF-induced genes. This is consistent with prior studies showing AMF association with roots enhances acquisition of phosphate and nitrogen and that AMF-root interactions are highly specific with well-defined stages of signaling, interaction, and association (Delaux et al., 2013; Gutjahr & Parniske, 2013).

### 4 DISCUSSION

Plant-root-system-mediated carbon sequestration in soil profiles improves soil fertility, water-holding capacity, and crop productivity while reducing atmospheric carbon dioxide levels. Assessment of rates of SOC deposition by energy crop root systems has focused previously on perennial grasses such as switchgrass, sugarcane, *Miscanthus x giganteus*, and restored prairie (Anderson-Teixera et al., 2009; Martinez-Feria & Basso, 2020). These studies indicate that switchgrass has the potential to add ~0.87 Mg C/ha/yr although substantial variation (0.2–2.0 Mg C/ha/yr) is predicted based on starting levels of SOC, residue return, soil type/depth, crop productivity, the growing environment, and other factors (Martinez-Feria & Basso, 2020). Substantial SOC depletion has occurred on annual cropland (Lal, 2004; Post & Kwon, 2000) that could be restored through deployment of deep rooting crops in combination with improved management (Zomer et al., 2017). Annual photoperiod sensitive forage “bioenergy” sorghum was found to increase SOC (Meki et al., 2013; Shahandeh et al., 2016). The rate of SOC accumulation will depend on the size, distribution, and functional characteristics of bioenergy sorghum hybrid root systems. Therefore, a goal of this study was to obtain information about bioenergy sorghum root system biomass accumulation and distribution in soil profiles that contributes to our understanding of SOC accumulation, improves modeling, and informs comparisons to other crops (Field et al., 2020; Gelfand et al., 2020; Martinez-Feria & Basso, 2020). Information about grain sorghum root systems that can grow >1 m deep was collected decades ago to assist in modeling water extraction from soil profiles and to help explain the drought resilience of grain sorghum (Robertson et al., 1993, 1993a; Robertson et al., 1993b).

Bioenergy sorghum root system development is similar to grain sorghum during the vegetative growth phase. Sorghum seedlings initially produce a primary root followed within 10 days by proliferation of nodal roots and their associated lateral roots that comprise the bulk of the adult plant root system (Blum et al., 1977; Singh et al., 2010). Root data collection in this study focused on development of the nodal root system starting 32 DAE following the transition from the juvenile to adult vegetative phase. The number of nodal roots increased rapidly from ~30 at 32 DAE to ~160 by ~124 DAE and then more slowly to ~175 nodal roots at 155 DAE. In grain sorghum, nodal root production and root growth occur during the vegetative growth phase, but cease shortly after anthesis which occurs in most grain genotypes between ~60 and 70 DAE. At 64 DAE, bioenergy sorghum contained ~80 nodal roots, and in contrast to grain sorghum, nodal root number, root system biomass, depth, and overall size continued to increase through 155 DAE. Continuous expansion of the root system occurs throughout the long growing season because bioenergy sorghum is highly photoperiod sensitive; therefore, plants remain vegetative until short day lengths occur late in the growing season delaying anthesis in our field location into mid to late October, typically ~150–175 DAE (Murphy et al., 2011, 2014). Root biomass accumulated in bioenergy sorghum root systems throughout the growing season to a final level of ~7 Mg/ha consistent with prior measurements of photoperiod sensitive.
### Table 3

Expression of sorghum genes encoding nitrate, ammonium, and phosphate transporters in bioenergy sorghum roots. Sorghum transcripts/genes were identified that are annotated as encoding nitrate, ammonium, or phosphate transporters based on identification of the closest *A. thaliana* homolog (A.th Best hit, Gene name, Function). The expression (TPM) of these genes in nodal, surface, and deep roots (highest TPM highlighted) along with Tau values

| Transcript ID | A.th. Best hit | Gene name | Function | Nodal roots | Surface roots | Deep roots | Tau |
|---------------|----------------|------------|----------|-------------|--------------|------------|-----|
| **Nitrate transport** | | | | | | | |
| Sobic.004G260500.1 | AT1G32450.1 | NRT1.5 | Nitrate transporter 1.5 | NR | 22 | 3 | 4 | 0.84 |
| Sobic.001G302800.1 | AT1G12110.1 | NRT1.1 | Nitrate transporter 1.1 | NR | 27 | 7 | 2 | 0.83 |
| Sobic.009G099000.1 | AT3G45650.1 | NAXT1 | Nitrate transporter1 | NR,SR | 8 | 6 | 2 | 0.50 |
| Sobic.004G009500.1 | AT5G60770.1 | NRT2.4 | Nitrate transporter 2.4 | SR | 0 | 75 | 2 | 0.99 |
| Sobic.004G009400.1 | AT5G60770.1 | NRT2.4 | Nitrate transporter 2.4 | SR | 0 | 62 | 2 | 0.99 |
| Sobic.004G202400.1 | AT5G50200.3 | NRT3.1 | Nitrate transporters | SR | 3 | 170 | 11 | 0.96 |
| Sobic.003G270800.1 | AT1G12940.1 | NRT2.5 | Nitrate transporter 2.5 | SR | 2 | 60 | 4 | 0.95 |
| Sobic.003G188200.1 | AT1G12940.1 | NRT2.5 | Nitrate transporter 2.5 | SR | 0 | 8 | 1 | 0.93 |
| Sobic.010G175900.2 | AT1G69850.1 | NRT1.2 | Nitrate transporter 1.2 | SR,DR | 1 | 15 | 25 | 0.67 |
| Sobic.001G541900.2 | AT1G12110.1 | NRT1.1 | Nitrate transporter 1.1 | SR,DR | 1 | 2 | 6 | 0.77 |
| Sobic.004G193000.1 | AT3G21670.1 | | Major facilitator protein | SR,DR | 3 | 19 | 27 | 0.58 |
| Sobic.007G044300.1 | AT1G12110.1 | NRT1.1 | Nitrate transporter 1.1 | AR | 111 | 46 | 55 | 0.54 |
| Sobic.005G107500.1 | AT1G69850.1 | NRT1.2 | Nitrate transporter 1.2 | AR | 21 | 15 | 12 | 0.37 |
| **Ammonium transport** | | | | | | | |
| Sobic.004G217800.1 | AT4G13510.1 | AMT1.1 | Ammonium transporter 1.1 | SR | 3 | 44 | 7 | 0.89 |
| Sobic.003G370400.1 | AT2G38290.1 | AMT2 | Ammonium transporter 2 | SR,DR | 0 | 162 | 61 | 0.81 |
| Sobic.006G143100.1 | AT4G13510.1 | AMT1.1 | Ammonium transporter 1.1 | SR,DR | 1 | 8 | 6 | 0.55 |
| Sobic.009G168700.1 | AT2G38290.1 | AMT2 | Ammonium transporter 2 | SR,DR | 1 | 20 | 23 | 0.54 |
| Sobic.004G173200.1 | AT2G38290.1 | AMT2 | Ammonium transporter 2 | SR,DR | 2 | 25 | 28 | 0.53 |
| Sobic.006G143100.2 | AT4G13510.1 | AMT1.1 | Ammonium transporter 1.1 | AR | 8 | 23 | 18 | 0.44 |
| Sobic.001G537500.1 | AT1G72660.3 | | Nucleoside triphosph. hydrolase | AR | 49 | 42 | 39 | 0.18 |
| **Phosphate transport** | | | | | | | |
| Sobic.004G199900.1 | AT3G26570.1 | PHT2.1 | Phosphate transporter 2.1 | NR | 61 | 1 | 4 | 0.96 |
| Sobic.003G133700.1 | AT2G29650.1 | PHT4.1 | Phosphate transporter 4.1 | NR | 8 | 1 | 1 | 0.82 |
| Sobic.010G080900.1 | AT5G14040.1 | PHT3.1 | Phosphate transporter 3.1 | NR | 39 | 10 | 11 | 0.73 |
| Sobic.002G293500.1 | AT5G20380.1 | PHT4.5 | Phosphate transporter 4.5 | NR | 12 | 4 | 3 | 0.72 |
| Sobic.006G026800.1 | AT5G43350.1 | PHT1.1 | Phosphate transporter 1.1 | SR | 0 | 17 | 2 | 0.95 |
| Sobic.001G502000.1 | AT5G47000.1 | PHT1.7 | Phosphate transporter 1.7 | SR | 2 | 8 | 0 | 0.90 |
| Sobic.007G045500.1 | AT3G47420.1 | PS3 | Phosphate starvation gene | AR | 8 | 24 | 8 | 0.67 |
| Sobic.001G054000.1 | AT3G52190.1 | PHF1 | Phos. transporter facilitator | SR,DR | 1 | 35 | 14 | 0.79 |
| Sobic.003G243400.1 | AT2G32830.1 | PHT5 | Phosphate transporter 1.5 | SR,DR | 0 | 194 | 106 | 0.73 |
| Sobic.002G3224100.1 | AT3G48850.1 | PHT3.2 | Phosphate transporter 3.2 | SR,DR | 2 | 17 | 26 | 0.62 |
| Sobic.001G234800.1 | AT2G38940.1 | PHT1.4 | Phosphate transporter 1.4 | AR,DR | 32 | 116 | 122 | 0.39 |
| Sobic.004G339800.2 | AT3G23430.1 | PHO1 | Phosphate 1 | AR,DR | 39 | 87 | 113 | 0.44 |
| Sobic.006G097400.1 | AT5G14040.1 | PHT3.1 | Phosphate transporter 3.1 | AR,DR | 4 | 9 | 13 | 0.48 |
| Sobic.004G310300.1 | AT5G14040.1 | PHT3.1 | Phosphate transporter 3.1 | AR | 383 | 252 | 246 | 0.35 |
| Sobic.006G173000.3 | AT3G47420.1 | PS3 | Phos. starvation-induced | AR | 8 | 7 | 3 | 0.34 |
| Sobic.009G131300.1 | AT5G23630.1 | PDR2 | Phos. deficiency response | AR | 56 | 39 | 65 | 0.27 |
| Sobic.001G169100.3 | AT5G44370.1 | PHT4.6 | Phosphate transporter 4.6 | AR | 6 | 6 | 3 | 0.28 |
“bioenergy” forage sorghum root biomass accumulation and crop modeling predictions (7.2–10.6 Mg/ha) (Meki et al., 2013; Shadandeh et al., 2016). SOC was not measured in the current study, but a 7-year study of a forage “bioenergy” sorghum showed that plots of continuous sorghum with ~2.4 Mg/ha residue return increased SOC substantially in the top 30 cm of the soil profile (~2.17 Mg C/ha/yr) (Shahandeh et al., 2016). A large portion of the bioenergy sorghum root biomass is located in large diameter nodal roots that emerge from stem nodes and extend into the top of the soil profile within 20 cm of the stem similar to grain sorghum (~80%) (Myers, 1980) and maize (Nichols et al., 2019).

A meta-analysis of root biomass accumulation showed that maize accumulated an average of ~31 g of root biomass per plant with a root:shoot ratio of ~0.15 (Amos & Walters, 2006). Maize is typically grown at 40,000–80,000 plants/ha providing an estimated accumulation of 1.2–2.5 Mg of root biomass/ha/yr. A more recent multi-location study of maize and soybean root biomass accumulation in the US Midwest found that depending on location, ~1.2 to 2.8 Mg of maize root biomass accumulated annually per hectare (Ordóñez et al., 2020). Maize root growth stops shortly after anthesis (~70 days after emergence) limiting the duration of root growth relative to bioenergy sorghum (~150–175 DAE) consistent with significantly more root biomass accumulation in bioenergy sorghum hybrids observed in this study (~7 Mg/ha). However, there are several factors that limit direct comparison of the extensive data on maize root systems to results obtained in the current study. First, the biomass of up to 25 aerial nodal roots that grow out from upper internodes of bioenergy sorghum later in the season were included in root biomass estimates, whereas aerial roots are not produced or not included in assessments of maize root biomass. The bioenergy sorghum aerial roots can accumulate ~0.5 Mg of root biomass per hectare. Aerial roots located highest on the stem are harvested with stems and would not contribute to restoration of SOC. Second, the current study focused on a single bioenergy sorghum hybrid (TX08001) grown in a location that has deep soils. Multi-location studies of a panel of bioenergy sorghum hybrids will be needed to better understand the extent and variation of root biomass production of this relatively new crop. Despite these limitations, a key finding of this study is that bioenergy sorghum root systems continue to grow deeper and accumulate biomass throughout a long growing season explaining the relatively high root biomass accumulation compared to early flowering maize or grain sorghum crops. Root system exudation and turnover occurs during the growing season so end of season biomass assessments significantly underestimate the total root biomass deposited in soil profiles during the season (Amos & Walters, 2006). The identification of roots comprised of endodermal an inner root tissues surrounded by cortical and epidermal cell layers that were filled with aerenchyma or fully degraded is consistent with substantial turnover of root cells during the growing season.

The importance of rooting depth for uptake of water, nutrients, and sequestration of carbon has been emphasized by many authors (Kell, 2011; Lynch & Wojciechowski, 2015; Thorup-Kristensen et al., 2020). The roots of grain sorghum can reach depths of 150 cm (Myers, 1980) to 190 cm (Robertson, Fukai, Hammer, et al., 1993; Stone et al., 2001) in soil profiles. A multi-location study of maize root systems found that maximum root system depth ranged from 89 to 157 cm and that root depth did not exceed the average water table (Ordóñez et al., 2018). In the current study, bioenergy sorghum roots were observed 2 m deep in clay-rich soil and >2.4 m deep in sandy soils late in the growing season. Deep deployment of bioenergy sorghum root systems is expected to enhance the acquisition of nutrients and water from the soil profile. In addition, SOC deposited by deep roots has been shown to turnover at a slower rate compared to roots near the soil surface (Balesdent et al., 2018). This may explain why in a 7-year trial of a bioenergy sorghum forage, SOC increased in the deepest portion of the soil profile measured (60–90 cm) (Shahandeh et al., 2016) even though root biomass accumulation at that depth is much less than the top 20 cm of the soil profile.

| Transcript ID | A.th. Best hit | Gene name | Function | Nodal roots | Surface roots | Deep roots | Tau |
|--------------|---------------|-----------|----------|-------------|--------------|-----------|-----|
| Sobic.003G358900.1 | AT3G46980.1 | PHT4.3 | Phosphate transporter 4.3 | AR | 20 | 13 | 18 | 0.25 |
| Sobic.009G157700.1 | AT2G38060.1 | PHT4.2 | Phosphate transporter 4.2 | AR | 12 | 19 | 18 | 0.21 |
| Sobic.001G428500.1 | AT5G14040.1 | PHT3.1 | Phosphate transporter 3.1 | AR | 9 | 13 | 12 | 0.20 |
| Sobic.002G060900.1 | AT3G32190.1 | PHF1 | Phos. transporter facilitator | AR | 12 | 12 | 9 | 0.18 |
| Sobic.008G151500.1 | AT5G35730.1 | EXS family protein | AR | 54 | 52 | 75 | 0.30 |
| Sobic.008G151366.1 | AT5G35730.1 | EXS family protein | AR | 14 | 11 | 12 | 0.19 |

| Nodal | Surface | Deep | Tau |
|-------|--------|------|-----|
| 20    | 13     | 18   | 0.25|
| 12    | 19     | 18   | 0.21|
| 9     | 13     | 12   | 0.20|
| 12    | 12     | 9    | 0.18|
| 54    | 52     | 75   | 0.30|
| 14    | 11     | 12   | 0.19|
The depth of bioenergy sorghum root growth into the soil profile early in the growing season could have been restricted by a high water table and low oxygen as suggested for maize (Ordóñez et al., 2018). However, later in the season, roots can grow deeper as the soil profile drains and water table is lower. Soil strength and impedance can also reduce root growth rates especially in soils with low water content (Gao et al., 2016). Grass roots penetrate high impedance soils by growing through macropores that range in diameter from ~30 to 300 μm (Jin et al., 2013; Kravchenko & Guber, 2017; Kravchenko et al., 2019; Valentine et al., 2012). Sorghum roots located deep in soil profiles at mid-row were ~30–500 μm in diameter. This range of root diameters appears ideally suited for growth through macropores and cracks present in high impedance soils. The root specific density (biomass per root volume) of large diameter nodal roots is 2–4 times greater than small diameter roots located more than 20 cm from plant stems and taking into consideration the large differences in root diameter, the biomass per unit root length is ~50–100 times lower for small diameter roots. Large diameter nodal roots occupy approximately ~5% of the soil profile, whereas small diameter (~0.3 mm) roots predominate in 95% of the 2 m deep soil profile, minimizing the overall energetic cost of the root system. The anatomy of nodal roots and small diameter lateral roots differed in terms of the location and number of metaxylem. Conversion of cortical cells to aerenchyma was observed in both root types leading in some roots to nearly total degradation of cell layers outside the endodermis. The remobilization of carbon and nitrogen from outer cortical cell layers of the root would reduce the investment of biomass in root system proliferation while retaining long-distance transport capacity. Further analysis of root exudation, root tissue and system turnover during the season is needed to obtain a more complete understanding of bioenergy sorghum root system developmental dynamics and response to soil environments.

In maize, root length density decreases with soil depth and distance from the stem to mid-row (Gao et al., 2010; Ordóñez et al., 2018). Bioenergy sorghum root length density is similar from the row to 19 cm from the row and then decreased 25% from 19 cm to 38 cm from the row. This more gradual decrease in root length density from row to mid-row could be a consequence of proliferation of bioenergy sorghum root systems over a longer growing season. Bioenergy sorghum and grain sorghum root length density decrease as a function of soil depth. Bioenergy sorghum root length density measured in this study is somewhat higher than reported for grain sorghum at all depths in the soil profile (Robertson, Fukai, Hammer, et al., 1993). This is consistent with continuous production of nodal roots and proliferation of their associated lateral root systems in the soil profile for ~155 days in bioenergy sorghum compared to ~70 days in grain sorghum. Quantitative estimates of the impact of differences in growing season length on root length density will require comparisons of grain sorghum, maize, and bioenergy sorghum grown in the same location and season.

Transcriptome analysis of recently embedded bioenergy sorghum nodal roots and surface and deep roots located 38 cm from the stem (mid-row) identified >2,500 genes/transcripts that were differentially expressed 10-fold or more in comparisons of the three root systems. The large number of differentially expressed genes was not surprising since the anatomy, functional specialization, and soil location of recently embedded nodal roots differ significantly from root systems comprised predominantly of lateral roots in surface and deep soil profiles at mid-row. Transcriptome analysis of primary, seminal, and crown roots of maize also revealed root-type-specific gene expression indicative of functional specialization (Tai et al., 2016). In bioenergy sorghum, genes differentially expressed in surface and deep roots were highly root specific with 60–90% of the genes having Tau values 0.9 or greater when the expression of these genes in roots was compared to shoot tissues (leaves, stems, panicles, seed). Genes differentially expressed in deep roots were homologous to genes known to regulate lateral root meristem activity and lateral root growth (i.e., WOX11, ATC, ATATM, bZIP29, ADA2B, PLAIVA, WRKY46/54/70). The identification of sorghum homologs of genes involved in IAA biosynthesis (YUCCA3) and signaling (PAP1) and ABA signaling (HIA2, AHG1) indicates these hormones help regulate the growth and adaptation of bioenergy sorghum roots to the deep soil environment. Sorghum homologs of genes involved in signaling (WAK2, WAK3), energy homeostasis (ADK), plant defense (i.e., DIR1, PR6, WIN2), and transport (i.e., SWEET11/12, AAP2) were also identified. Genes differentially expressed in surface roots included homologs of genes involved in nitrogen/nutrient signaling (i.e., HAT14, RAP2.11, NF-NYC2) and growth regulation (i.e., AHB1, PLD1, GL22) and a large number of genes involved in plant defense and/or plant–microbe interaction.

The capacity of bioenergy sorghum roots to take up and transport nitrogen, phosphate, and other nutrients contributes to the low input requirements of this crop. Analysis of genes annotated as transporters identified five sorghum genes involved in nitrate transport, two phosphate transporters, and transporters for aluminum, boron, and iron in surface roots. Moreover, two nitrate transporters were differentially expressed in nodal roots, three in surface and deep roots, and two genes encoding nitrate transporters were expressed in all three root systems. In contrast, four ammonium transporters were differentially expressed in both surface and deep roots and only one was differentially expressed in surface roots. Differential expression of genes involved in
nitrate transport and assimilation (i.e., nitrate reductase, nitrite reductase) in surface roots is consistent with addition of nitrate fertilizer to the soil surface at the beginning of the season. Expression of ammonium transporters in surface and deep roots indicates this ion is available throughout the soil-root profile. Expression of phosphate transporters was also complex with four genes differentially expressed in nodal roots and three genes differentially expressed in surface roots. The information on differential gene expression collected in this study provides the starting point for determining the subcellular location, function, and regulation of specific genes involved in growth and nutrient uptake/transport in bioenergy sorghum roots. This is especially important to reduce nitrous oxide emissions early in the growing season as consequence of addition of N-fertilizer in excess of bioenergy sorghum requirements and the capacity and deployment of developing root systems to take up nutrients (Moore et al., 2021; Storlien et al., 2014). Moreover, the availability of nitrogen was previously found to alter soil microbe profiles and compromise salicylic acid biosynthesis (Sheflin et al., 2019). Bioenergy sorghum has high nitrogen use efficiency (NUE) (Olson et al., 2013) and relatively low but not fully characterized N-fertilizer requirements (Maw et al., 2019) due to C4 photosynthesis, high radiation use efficiency, long duration of vegetative growth, and efficient nitrogen recycling within the canopy (Byrt et al., 2011; Olson et al., 2013). Sorghum root systems are associated with nitrogen fixing bacteria (Hara et al., 2019) and arbuscular mycorrhizal fungi (AMF) (Watts-Williams et al., 2019) that facilitate the acquisition of nitrogen and phosphate from soils. Deep sorghum roots express genes that encode transporters for nitrate, ammonium, phosphate, micronutrients, and water and are ideally positioned to remove excess N-fertilizer leached below the root zone of prior annual crops. Taken together, we predict that the N-fertilization requirements for bioenergy sorghum and nitrous oxide emissions associated with growing this crop will be lower than the values used recently to calculate bioenergy sorghum C.I. (Kent et al., 2020).

Roots located in close proximity to grain sorghum stems were subject to transcriptome analysis during development and water deficit in the field (Varroquaux et al., 2019). Expression of genes known to be induced by interaction of AMF with roots (Watts-Williams & Cavagnaro, 2018) was decreased in response to soil drying (Varroquaux et al., 2019). In the current study, most of the ~300 sorghum genes previously identified as AMF inducible (Watts-Williams & Cavagnaro, 2018) were differentially expressed in both surface and deep roots relative to nodal roots and above ground shoot tissues. This indicates that AMF–sorghum root interactions occur throughout the 2 m deep soil profile potentially contributing to uptake of phosphate and nitrogen (Cavagnaro et al., 2015; Harrison, 2012). AMF-facilitated uptake of nutrients is consistent with differential expression of one ammonium transporter and two phosphate transporters that were previously found to be induced specifically by AMF–root interaction (Watts-Williams & Cavagnaro, 2018). Since sorghum roots interact with a wide range of microbes and these interactions are modulated by environmental conditions (Dhawi et al., 2016; Rout & Chrzanowski, 2009; Schlemper et al., 2018; Xu et al., 2018), a more complete understanding of the dynamics and benefits of bioenergy sorghum root–microbiome–environment interaction is warranted.

Crop models have been developed to help predict bioenergy sorghum crop yield under non water-limiting conditions and in response to regional historical variation in rainfall (Truong et al., 2017) to simulate variation in biomass yield and changes in soil organic matter under bioenergy forage sorghum production (Dou et al., 2014). Modeling has also been used to assess the impact of bioenergy sorghum on strategies for scale-up of cellulosic biofuel production (Cui et al., 2018) and to predict biomass yield of sorghum under future climate scenarios (Hoffman et al., 2020; Huntington et al., 2020). The current study was focused on collecting information about the bioenergy sorghum root system that could help enhance the accuracy of root system modeling and the potential impact of this annual bioenergy crop on SOC. There are millions of acres of abandoned or marginal cropland in the United States and worldwide (Cai et al., 2011; Zumkehr & Campbell, 2013) as well as within-field locations (Maestrini & Basso, 2018) that provide minimal return on investment when planted with grain crops (Bonne et al., 2014; Brandes et al., 2016). This portion of annual cropland could be used to grow bioenergy sorghum due to the crop’s high water use efficiency and drought resilience. The deployment of bioenergy sorghum in these target regions of production could provide a significant amount of biomass for biofuels production especially in the gulf coastal region (Cai et al., 2011). The combination of biorefinery-associated carbon capture and sequestration (Sanchez et al., 2018) and plant-root-based accumulation of SOC has the potential to restore the fertility of marginal annual cropland soils that have been depleted of carbon over the past 100 years and substantially mitigate transportation fleet derived carbon dioxide emissions.

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**DATA AVAILABILITY STATEMENT**

Data pertaining to Figures 1–4, Figure 1, and Table S3 have been deposited online at: Austin Lamb, John Mullet, and Brock Weers. 2021. Bioenergy Sorghum’s Deep Roots—A Key to Sustainable Biomass Production on Annual Cropland—Data set. Knowledge Network Deep Roots—A Key to Sustainable Biomass Production S3 have been deposited online at: Austin Lamb, John e40e8f921078. Expression data, pertaining to all other figure and tables, are deposited on the Joint Genome Institute Genomicus Portal under the project name: Sorghum bicolor TX08001 Nodal root development Gene Expression Profiling (Project ID: 1208513).

**ORCID**

Austin Lamb @ https://orcid.org/0000-0003-0083-637X

**REFERENCES**

Amos, B., & Walters, D. T. (2006). Maize root biomass and net rhizodeposited carbon. *Soil Science Society of America Journal*, 70(5), 1489–1503. https://doi.org/10.2136/sssaj2005.0216

Anders, S., & Huber, W. (2010). Differential expression and sequence-specific interaction of karyopherin α with nuclear localization sequences. *Genome Biology*, 11, R106. https://doi.org/10.1186/gb-2010-11-10-r106

Anderson-teixeira, K. J., Davis, S. C., Masters, M. D., & Delucia, E. H. (2009). Changes in soil organic carbon under biofuel crops. *GCB Bioenergy*, 1(1), 75–96. https://doi.org/10.1111/j.1757-1707.2008.00101.x

Arnh, A., Barbosa, H., Benton, T., Calvin, K., Calvo, E., Connors, S., Cowie, A., Davin, E., Denton, F., van Diemen, R., Fatima, D., Elbehri, A., Evans, J., Ferrat, M., Harald, J., Haughey, E., Herrero, M., House, J., Howden, M., ... Zommers, Z. (2019). Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems. In *Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems.*

Batjes, N. H. (2000). Total carbon and nitrogen in the soils of the world. *ASAIO Journal*, 46(4), 378. https://doi.org/10.1097/0002480-200007000-00002

Blum, A., Arkin, G. F., & Jordan, W. R. (1977). Sorghum root morphogenesis and growth. I. Effect of maturity genes 1. *Crop Science*, 17(1), 149–153. https://doi.org/10.2135/cropsci1977.001183x00170010039x

Bonner, I. J., Cafferty, K. G., Muth, D. J., Tomer, M. D., James, D. E., Porter, S. A., & Karlen, D. L. (2014). Opportunities for energy crop production based on subfield scale distribution of profitability. *Energies*, 7(10), 6509–6526. https://doi.org/10.3390/en7106509

Brandes, E., McNunn, G. S., Schulze, L. A., Bonner, I. J., Muth, D. J., Babcock, B. A., Sharma, B., & Heaton, E. A. (2016). Subfield profitability analysis reveals an economic case for cropland diversification. *Environmental Research Letters*, 11(1), https://doi.org/10.1088/1748-9326/11/1/014009

Brumbarova, T., & Ivanov, R. (2019). The nutrient response transcriptional Regulome of Arabidopsis. *Isicience*, 19, 358–368. https://doi.org/10.1016/j.isi.2019.07.045

Byrt, C. S., Grof, C. P. L., & Furbank, R. T. (2011). C4 plants as biofuel feedstocks: Optimising biomass production and feedstock quality from a lignocellulosic perspective. *Journal of Integrative Plant Biology*, 53(2), 120–135. https://doi.org/10.1111/j.1744-7909.2010.01023.x

Cai, X., Zhang, X., & Wang, D. (2011). Land availability for biofuel production. *Environmental Science and Technology*, 45(1), 334–339. https://doi.org/10.1021/es103338e

Cavagnaro, T. R., Bender, S. F., Asghari, H. R., & van der Heijden, M. G. A. (2015). The role of arbuscular mycorrhizas in reducing soil nutrient loss. *Trends in Plant Science*, 20(5), 283–290. https://doi.org/10.1016/j.tplants.2015.03.004

Chen, J., Nolan, T. M., Ye, H., Zhang, M., Tong, H., Xin, P., Chu, J., Chu, C., Li, Z., & Yina, Y. (2017). Arabidopsis WRKY46, WRKY54, and WRKY70 transcription factors are involved in brassinosteroid-regulated plant growth and drought responses. *The Plant Cell*, 29(6), 1425–1439. https://doi.org/10.1105/tpc.17.00364

Cho, M., Lee, Z. W., & Cho, H. T. (2012). ATP-binding cassette B4, an auxin-efflux transporter, stably associates with the plasma membrane and shows distinctive intracellular trafficking from that of PIN-FORMED proteins. *Plant Physiology*, 159(2), 642–654. https://doi.org/10.1104/pp.111.196139

Creelman, R. A., Mason, H. S., Bensen, R. J., Boyer, J. S., & Mullet, J. E. (1990). Water deficit and abscisic acid cause differential inhibition of shoot versus root growth in soybean seedlings: Analysis of growth, sugar accumulation, and gene expression. *Plant Physiology*, 92(1), 205–214. https://doi.org/10.1094/pp.92.1.205

Cruzen, P. J., Mosier, A. R., Smith, K. A., & Winiwarter, W. (2008). N2O release from agro-biofuel production negates global warming reduction by replacing fossil fuels. *Atmospheric Chemistry and Physics*, 8, 389–395. https://doi.org/10.5194/acp-8-389-2008

Cui, X., Kavvada, O., Huntington, T., & Scown, C. D. (2018). Strategies for near-term scale-up of cellulose biofuel production using sorghum and crop residues in the US. *Environmental Research Letters*, 13(12), 124002. https://doi.org/10.1088/1748-9326/aae663

Daehwan, K., Langmead, B., & Salzberg, S. L. (2015). HISAT: A fast spliced aligner with low memory requirements. *Nature Methods*, 12(4), 357–360. https://doi.org/10.1038/nmeth.3317

Davies, J. (2017). The business case for soil. *Nature*, 543, 309. https://doi.org/10.1038/543309a
Yu, P., Gutjahr, C., Li, C., & Hochholdinger, F. (2016). Genetic control of lateral root formation in cereals. *Trends in Plant Science*, 21(11), 951–961. https://doi.org/10.1016/j.trendsplant.2016.07.011

Zhang, L., Tan, Q., Lee, R., Trethewy, A., Lee, Y. H., & Tegeder, M. (2010). Altered xylem-phloem transfer of amino acids affects metabolism and leads to increased seed yield and oil content in Arabidopsis. *The Plant Cell*, 22(11), 3603–3620. https://doi.org/10.1105/tpc.110.073833

Zhao, J. (2015). Phospholipase D and phosphatic acid in plant defence response: From protein-protein and lipid-protein interactions to hormone signalling. *Journal of Experimental Botany*, 66(7), 1721–1736. https://doi.org/10.1093/jxb/eru540

Zhu, L., Xin, R., Bu, Q., Shen, H., Dang, J., & Huq, E. (2016). A negative feedback loop between PHYTOCHROME INTERACTING FACTORs and HECATE proteins fine-tunes photomorphogenesis in Arabidopsis. *The Plant Cell*, 28(4), 855–874. https://doi.org/10.1105/tpc.16.00122

Zomer, R. J., Bossio, D. A., Sommer, R., & Verchot, L. V. (2017). Global sequestration potential of increased organic carbon in cropland soils. *Scientific Reports*, 7(1), 1–8. https://doi.org/10.1038/s41598-017-15794-8

Zumkehr, A., & Campbell, J. E. (2013). Historical U.S. cropland areas and the potential for bioenergy production on abandoned croplands. *Environmental Science and Technology*, 47(8), 3840–3847. https://doi.org/10.1021/es3033132

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