

Root System Architecture and Environmental Flux Analysis in Mature Crops using 3D Root Mesocosms

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1 Abstract

2 Current methods of root sampling typically only obtain small or incomplete sections of root systems
3 and do not capture their true complexity. To facilitate the visualization and analysis of full-sized
4 plant root systems in 3-dimensions, we developed customized mesocosm growth containers. While
5 highly scalable, the design presented here uses an internal volume of 45 ft³ (1.27 m³), suitable for
6 large crop and bioenergy grass root systems to grow largely unconstrained. Furthermore, they allow
7 for the excavation and preservation of 3-dimensional RSA, and facilitate the collection of time-
8 resolved subterranean environmental data. Sensor arrays monitoring matric potential, temperature
9 and CO₂ levels are buried in a grid formation at various depths to assess environmental fluxes at
10 regular intervals. Methods of 3D data visualization of fluxes were developed to allow for comparison
11 with root system architectural traits. Following harvest, the recovered root system can be digitally
12 reconstructed in 3D through photogrammetry, which is an inexpensive method requiring only an
13 appropriate studio space and a digital camera. We developed a pipeline to extract features from the
14 3D point clouds, or from derived skeletons that include point cloud voxel number as a proxy for
15 biomass, total root system length, volume, depth, convex hull volume and solidity as a function of
16 depth. Ground-truthing these features with biomass measurements from manually dissected root
17 systems showed a high correlation. We evaluated switchgrass, maize, and sorghum root systems to
18 highlight the capability for species wide comparisons. We focused on two switchgrass ecotypes,
19 upland (VS16) and lowland (WBC3), in identical environments to demonstrate widely different root
20 system architectures that may be indicative of core differences in their rhizoeconomic foraging
21 strategies. Finally, we imposed a strong physiological water stress and manipulated the growth
22 medium to demonstrate whole root system plasticity in response to environmental stimuli. Hence,
23 these new “3D Root Mesocosms” and accompanying computational analysis provides a new
24 paradigm for study of mature crop systems and the environmental fluxes that shape them.

25 1 Introduction

26 A plant's root system is a complex set of organs that do more than simply anchor the plant to the
27 ground and provide paths of uptake from the soil (Calvo et al., 2020; Novoplansky, 2019). Roots
28 allow a plant to perceive its surroundings and adjust future growth accordingly, maximizing its

Root System Architecture and Environmental Flux Analysis in Mature Crops using 3D Root Mesocosms

29 chances of survival and reproduction (Bao et al., 2014; Dowd et al., 2019, 2020; Galvan-Ampudia
30 and Testerink, 2011; Hématy et al., 2009; Knight, 1811; O'Brien et al., 2016). Thus, a plant's Root
31 System Architecture (RSA) is highly adaptable and is strongly affected by water and nutrient
32 availability, competition with neighbors, rhizosphere interactions, and other aspects of the local
33 growth environment (Gruber et al., 2013; Malamy, 2005; Morris et al., 2017; Rogers and Benfey,
34 2015; Yu et al., 2014). While it is widely accepted that understanding root form and function is one
35 of the most critical aspects of plant biology, very little is known about below ground traits such as
36 RSA compared to the wealth of information on above ground plant structures. As subterranean
37 tissues with complex architectures that branch exponentially over time, they are very difficult to
38 completely characterize, especially deep underground. Many methods exist to study root system
39 architecture in various growth environments and growth stages (Atkinson et al., 2019; Dowd et al.,
40 2021). However, all methods have significant tradeoffs, leading to the well-known gap between the
41 information-dense data sets captured from plants grown in controlled environments, and the more
42 realistic, but information-sparse nature of measurements collected from plants in the open field
43 (Poorter et al., 2016; Topp et al., 2016).

44 Here we report the adaptation of traditional "root mesocosms" as a bridging system to facilitate the
45 growth, excavation, and preservation of 3-dimensional (3D) RSA, while providing the unconstrained
46 growth available in the field (Dowd et al., 2021; Odum, 1984). We incorporated sensor arrays to
47 measure biologically relevant gradients and dynamics of environmental factors: matric (water)
48 potential, temperature, and sub-soil CO₂ content at various depths in the soil profile. We modeled the
49 3D environmental data to facilitate the comparison of the environmental conditions over time with
50 the RSA, which in the future could be used for *post hoc* predictions of root activity and plasticity.
51 Using photogrammetry (aka Structure from Motion, SfM), we generated highly detailed 3D
52 reconstructions of the root systems and developed a pipeline for analysis across the soil profile.
53 Accuracy of the 3D models was verified using manual ground truthing in 3D space. Clear differences
54 among grass species RSA and in the effects of ecotypes and environments on RSA were measured as
55 a demonstration of the flexibility and power of the approach.

56

57 2 Materials & Methods

58 2.1 Mesocosm construction and preparation

59 The mesocosm is composed of several subsystems: 1) The external frame, 2) the internal frame, and
60 3) the sensors. Additional equipment is needed to digitize, visualize and analyze the RSA
61 information.

62 The external frame has a base constructed using pressure treated 10.2 x 10.2 cm dimensional lumber
63 (i.e. 4x4s). The unit has a foot print of 135.3 cm x 109.9 cm. Four of the 4x4 pieces measuring 135.3
64 cm long are laid out parallel to each other, with one on each outside edge and two in the middle with
65 a spacing of 10.8 cm. This configuration allows a standard pallet jack or forklift to pick up the unit.
66 Two 4x4s measuring 109.9 cm are attached on top of the existing 4x4s using galvanized 1.9 cm bolts
67 at each end, running perpendicular to create the rectangular base. Five 5.1 x 15.2 cm pressure treated
68 yellow pine dimensional lumber boards (i.e. 2x6s) cut to a length of 109.9 cm were then laid out
69 parallel to the top 4x4 boards, and attached to the first four 4x4 boards using 7.6 cm construction
70 screws, thus creating a base for the mesocosm unit (Fig 1A). Four 4x4s that were 182.9 cm in length

Root System Architecture and Environmental Flux Analysis in Mature Crops using 3D Root Mesocosms

71 were inserted with a vertical orientation at the inside of the four-perimeter base frame. These
72 vertically oriented 4x4s were attached using two 1.9 cm galvanized bolts. A drain box was
73 constructed using 1.9 cm thick plywood that was constructed with an interior dimension of 91.5 cm x
74 91.5 cm (Fig 1B). The drain box was centered on the external frame base between the four vertical
75 4x4s. This box was lined with a polyvinyl pond liner and fitted with a 1.9 cm diameter drain pipe
76 which stuck out the front of the mesocosm unit. The box was then filled with stones ranging from
77 one to 7.6 cm diameter and an expanded metal top was placed on it.

78 The internal frame is used to support the roots, and maintain their spatial configuration when the
79 roots are removed from the unit at the end of the experiment. The internal frame is constructed using
80 a 1.3 cm nominal diameter polyvinyl chloride (PVC) pipe. The internal frame is a rectangular prism
81 152 cm tall consisting of 10 layers with each layer being 15.3 cm apart. Each layer of the frame is
82 square in shape with a nominal length of 91.4 cm. Each side of the square has a 0.32 cm hole drilled
83 at 10 cm increments. A polycarbonate line is strung across the frame connecting opposite holes, thus
84 creating a 10 cm x 10 cm grid in the XY plane. When these squares are assembled together it creates
85 a 10 cm x 10 cm x 15.3 cm grid in the XYZ planes (Fig 1C).

86 The drain pipe was used to designate the front of the unit. Eight 2x6 dimensional boards were used to
87 connect the front vertical 4x4s on both the left and the right side. These 2x6s were attached on the
88 inside face of the 4x4's using 17.6 cm long construction screws, leaving 7.6 cm gaps between boards.
89 The front and back of the unit had seven 2x6s connecting the left side to the right side. These boards
90 were connected to the outside face of the 4x4 using galvanized 1.9 cm nuts and bolts, which allowed
91 the boards to be taken on and off as needed (Fig 1D).

92 Once the external frame is constructed, four sheets of 0.3 cm thick particle board are cut to 121.9 cm
93 long by 91.5 cm wide. These boards are placed with the long side in the vertical orientation, and on
94 the inside of the external frame. A 16 mil (0.4 mm) thick polyethylene tarp is folded using an origami
95 technique to create a rectangular prism shape that matches the external frame. The internal frame was
96 then placed inside the tarp, and the front of the mesocosm unit was closed up (Fig 1E).fig

97

98 **2.2 Environmental monitoring**

99 **2.2.1 Matric potential, temperature and CO₂ sensors**

100 A variety of sensor arrays have been tested and deployed in the mesocosms system. Three metrics
101 that have successfully been modeled to capture their dynamics in 3D space are the matric potential
102 and temperature of the growth media as well as sub-soil CO₂ levels. Temperature and matric
103 potential are both measured via TEROS21 (Meter Group Inc., Pullman, WA, USA) sensors
104 connected to Em50 data loggers (Meter Group Inc., Pullman, WA, USA) while CO₂ measurements
105 were taken using a Picarro G2201-i Isotopic Analyzer (Picarro Group, Santa Clara, CA, USA). By
106 arranging the sensors in an array of 14 sampling points throughout the growth volume data
107 interpolations allow the 3D modeling of the dynamic fluxes in the root system's local growth
108 environment (Fig 2). Matric potential and temperature measurements were set to record hourly,
109 continuously. The CO₂ profile throughout the growth volume was assessed by sampling air from
110 rubber tubes buried in an array. CO₂ measurements for each location in a mesocosm were sampled
111 for 10 minutes and the mean value of the recorded CO₂ levels were taken once weekly. Automation

Root System Architecture and Environmental Flux Analysis in Mature Crops using 3D Root Mesocosms

112 of sampling was facilitated using 14 ports on the Picarro 16-Port Distribution Manifold, set to switch
113 through sample ports connected to each tube in the array.

114

115 **2.2.2 Data interpolation**

116 We augment the 14 sensor data points (black points in Fig S1) to 35 data points by linearly
117 calculating the data at the 21 additional locations on the boundary of the 3D mesocosm box (purple
118 points in Fig S1). Note that we limit the maximum value of the water potential value to 0. These 21
119 boundary data points serve as boundary constraints for the 3D linear interpolation to the entire 3D
120 mesocosm growth volume. This 3D linear interpolation is conducted by running the MATLAB
121 function `griddatan()` which is a Delaunay triangulation based method.

122

123 **2.3 Mesocosm Harvest**

124 When the desired plant growth stage has been reached the mesocosms are prepared for harvest by
125 shutting down all irrigation and removing all the associated components (Fig 3A). If the experimental
126 design allows, it is beneficial to allow the mesocosms to dry for a few days before harvesting to ease
127 growth media extraction. At this time all cables from sensors are disconnected from data loggers and
128 the final data points are downloaded.

129 At harvest, shoot tissues of the samples are harvested by cutting the plants near the surface of the
130 growth media, above where the highest crown or adventitious root has emerged (Fig 3B). Shoot
131 tissues are bundled together and are dried down to obtain biomass measurements (Fig 3C) to
132 accompany any other shoot morphological data that was monitored during growth, such as plant
133 height or tiller production (Fig S2).

134 In the absence of any shoot-born roots, it is still important to have a section of plant tissue above the
135 growth media line to maintain proper orientation of the root system. Prior to the excavation of the
136 root system the sample must be tied in place to maintain its position after the removal of the growth
137 media. Additional fishing line, or other forms of support structures, can be used to tie the tissue
138 emerging from the growth media surface (base of the shoot/ top of the root crown) to the top most
139 section of the PVC frame. These supports go underneath the crown at the same height as the growth
140 media and support the structure at the elevation it was at during growth. Tying the tissue off to all 4
141 sides will maintain the root crowns' location in the X and Y orientations.

142 After securing the sample each of the eight 2x6 boards on the front and back of the mesocosm are
143 loosened slightly to allow the removal of the particle board support on the front and back walls. Next
144 the top pair of 2x6 boards are removed to expose the interior of the mesocosm and allow access to the
145 uppermost layer of the growth media (Fig 3D). When excavating it is important to do so slowly as to
146 not damage, or sever, unseen roots. During excavation, gentle vacuum suction is applied from the
147 bottom of the exposed growth medium. This allows newly exposed roots to settle downward on the
148 nearest segments of the interior scaffold to maintain root architecture. Caution must be taken to
149 assure that the location of the vacuum tube is not in contact with any roots, direct suction can pull
150 them from their location or cause them to snap. If rooting is too dense then manual hand clearing is
151 necessary to excavate the section of the root system.

Root System Architecture and Environmental Flux Analysis in Mature Crops using 3D Root Mesocosms

152 It is also important not to harvest too deeply in any given section as a shift in the growth media could
153 lead to a landslide effect shearing roots in the process. This is more likely to happen if the growth
154 media is wet and has high cohesion. Accordingly, the section of the growth media column that was
155 exposed should be excavated completely before the next set of 2x6 boards are removed and the
156 process repeats. If the mesocosm being harvested has sensors arrayed throughout the growth media,
157 then each sensor is removed as they are excavated (Fig 3E). When the root tips of the deep axial roots
158 are fully exposed, then less delicate methods of medium removal, such as handheld shovels, can be
159 utilized to complete the excavation (Fig 3F).

160 After all of the growth media has been removed, the PVC frame can be slid out from the wooden
161 exterior to provide 360 access to the exposed root system (Fig 3G). Depending on the growth
162 medium used, an additional round of cleaning may be required to remove particles from dense areas
163 of the root system. The now clean and free-standing root system can be stored for future analysis.

164

165 **2.4 Photogrammetry**

166 Utilizing 2D-photographs to develop a 3D point cloud through photogrammetry is a low-cost process
167 requiring only a digital camera, an appropriate imaging studio, and photogrammetry software.
168 Photogrammetry software identifies and utilizes a vast number of unique identification markers in
169 each image to orient the photos in 3D space that share common markers. These can be natural/
170 architectural markers such as wood grain or lines between boards or bricks; or can be produced for
171 the purpose of being a positional marker, such as painted shapes or specific computer-generated
172 alignment patterns.

173 Our photogrammetry studio uses a combination of painted shapes (splatters and stencils), computer
174 generated markers (code available on OpenCV:
175 https://docs.opencv.org/4.x/d2/d1c/tutorial_multi_camera_main.html), and physical structures (AC
176 unit, electrical control box, wire conduits, etc.) (Fig 4A). This studio has also been outfitted with
177 many LED lights with very high color rendering indices and color temperatures of 5000K (daylight)
178 to capture the most color accurate images possible. In the studio the sample is positioned in a central
179 location between the lights to allow for full 360° movement around that sample and to minimize
180 shadows (Fig 4B)

181 When imaging a sample by hand it is critical to use a sufficiently high shutter speed to ensure that the
182 photographs of the sample and the environment remain in crisp focus. The images collected to
183 produce the photogrammetry analyses detailed in this manuscript were taken at a shutter speed of 125
184 on a Canon EOS 50D (Ōta, Tokyo, Japan) set on the Tv (Time-value) priority setting. Ideally a fairly
185 high aperture is also maintained to keep the entire root sample and identification markers within the
186 depth of focus range. We found an aperture of 11-14 was ideal for the photogrammetry studio used in
187 this study.

188 The zoom on the camera must not vary between images, as this will lead to artifacts in the resultant
189 point cloud or failure of the photogrammetry software. Images were captured using a 10-18 mm wide
190 angle lens with the zoom kept at 18 mm. It is important to keep the camera level during imaging
191 which is monitored by an attached bubble level on the top of the camera.

192 It is critical that nothing is moved while the imaging is taking place. If an object in the environment
193 (light plug cable, ground lights) or the sample itself is moved it will cause artifacts in the

Root System Architecture and Environmental Flux Analysis in Mature Crops using 3D Root Mesocosms

194 photogrammetry software. A small disturbance to the root scaffold will cause the very delicate roots
195 to swing back and forth and it is likely that noise will be introduced into the point cloud. This could
196 lead to a minor artifact, or possibly an entire doubling of the root system where two separate point
197 clouds of the sample are produced with a slight offset.

198 The first images are taken along the perimeter of the photogrammetry room at a minimum of 4
199 elevations (eye level, chest, waist, and knees). This is to obtain a good baseline of the room and the
200 ID markers (Fig 4C). This step will increase the match points of the up-close sample images and
201 assist in camera alignment. Following this, images will be moved forward to be much closer to the
202 plant sample. When imaging the sample up close photographs need to be captured on all sides as well
203 as top-down images that angle smoothly from a dome shape to the flat walls of the root system
204 scaffold. Images should have at least an 80% overlap (more is better) and should create a “dome” of
205 coverage surrounding the sample (Fig 4D). A full-size crop plant root system is typically 4000+
206 images (including the images of the room perimeter).

207 Following image collection, the 2D-photographs are imported to a photogrammetry software to
208 generate a 3D point cloud. The photogrammetry software found to perform the best with thin root
209 structures is Pix4Dmapper (Pix4D S.A. Prilly, Switzerland). During the photogrammetric process
210 voxels are mapped onto a 3D space to generate a 3D point cloud model of the sample. Once the point
211 cloud is produced, many existing algorithms and processes utilized for X-ray CT or MRI data can be
212 modified to analyze the point cloud and extract root system architectural traits.

213

214 **2.5 Semiautomated segmentation of the root system point cloud**

215 Following photogrammetry, the point cloud of the studio and surrounding environment is segmented
216 away from the portion of the point cloud representing the root system. The 3D point cloud of the root
217 system with the scaffold (white PVC pipes and green fishing lines) is loaded into Matlab (R2017a).
218 Each point has its (x,y,z) coordinates and (R,G,B) color information. We segment out the root system
219 from the point cloud by the following four steps.

220 **2.5.1 Linear transformation by aligning the scaffold point cloud to a predefined reference** 221 **model**

222 The first step standardizes the scaffold scale and position which is useful to remove the scaffold and
223 extract features, especially the vertical distribution. We selected eight points from the 3D point cloud
224 plotted in Matlab as target points. Four of these eight points are picked from the crossings of the
225 fishing line grid on the top layer. The other four are chosen from the bottom layer (red points in Fig
226 5A). To be able to visualize and select the points more easily, we only work on the local layer
227 containing the target points (middle panel in Fig 5A). The reference model is defined based on the
228 scaffold design. Then the control points on the reference model are set (right panel in Fig 5A). Note
229 these eight target points can be arbitrarily selected as long as they are not on the same plane and the
230 control points correspond correctly. A Procrustes alignment is performed to determine a linear
231 transformation (translation, rotation and reflection, scale) based on the target points and control
232 points. We then apply these components to transform the entire 3D point cloud.

Root System Architecture and Environmental Flux Analysis in Mature Crops using 3D Root Mesocosms

233 **2.5.2 Removal of the scaffold**

234 Although the scaffold now is aligned with the reference model (left panel in Fig 5B), we cannot
235 simply delete the points along the reference as roots could be in contact with or be growing along the
236 scaffold. Therefore, we determine the scaffold points not only based on the position, but also on the
237 color. We set a small neighbor region near the reference model in case the candidate scaffold is
238 slightly misaligned with the reference. We then define color thresholds to remove non-root points
239 such as white, gray, and green points (right panel in Fig 5B).

240 **2.5.3 Removal of background noise**

241 Additionally, it is likely that some blue color from the photo studio background could be merged into
242 the point cloud during the 3D reconstruction. We would like to remove the blue noise. We convert
243 the RGB color into CIELAB ($L^*a^*b^*$) color in which L^* represents lightness, a^* represents green to
244 magenta, b^* represents blue to yellow. We used a practical threshold ($b^* = 15$) which separates the
245 blue noise with the root (left panel in Fig 5C). The output of this process is a point cloud devoid of
246 artifactual color noise and natural in appearance (right panel in Fig 5C). However, it may still contain
247 some noise for various reasons, such as light refraction through the translucent fishing line giving it a
248 color similar to the surrounding roots. At this point the root system point cloud is saved as a .ply for
249 the manual post-cleaning process.

250 **2.5.4 Manual post-process cleaning**

251 Once the point cloud has gone through segmentation in MATLAB, the data is further cleaned to
252 remove unwanted artifacts, such as residual fishing line and noise. We make these changes in
253 CloudCompare (v2.11.1 (Anoia), 2022) where the image can be cleaned using precise segmentation.
254 Post-process manual cleaning allows for better accuracy of the root structure and can drastically
255 improve the clarity of the 3D root system model (Fig 5D).

256 Noise on the point cloud at this stage is common, such as artifactual points in a cloud system that are
257 not in the proximity of other roots, or remaining color transferred from the studio environment that
258 was not completely removed by the color thresholding. Furthermore, due to the structural
259 methodology of the mesocosm, it is necessary to remove certain artifacts from the point cloud that
260 remain after segmentation, such as remnants of the PVC and fishing line scaffold. The segmentation
261 tool is used to remove the noise and remnants, leaving an isolated root system (Fig S3).

262 Additionally, some areas of the point cloud will need manual correction and shaping. This is utilized
263 predominantly in locations where tape has been placed to keep the roots together if they have broken
264 during harvest or storage. The taped area appears larger and a different color in the point cloud but
265 can be shaved down using precise segmentation. Shaping and smoothing can also eliminate areas of
266 noise or unwanted artifacts. Once all artifacts are removed, the point cloud can be used for trait
267 extraction and skeletonization (Fig 5E).

268

269 **2.6 Root trait extraction from point clouds**

270 From the point cloud, we can directly measure some global traits such as total number of points,
271 convex hull volume (the volume of the smallest convex set containing the point cloud), elongation
272 (PCA on point cloud, taking the ratio between PC2 variance and PC1 variance), flatness (the ratio

Root System Architecture and Environmental Flux Analysis in Mature Crops using 3D Root Mesocosms

273 between PC3 variance and PC2 variance), and maximum depth (the depth of deepest root point). We
274 also can measure the vertical distributions for biomass (Gaussian density estimator for point cloud),
275 convex hull volume (Gaussian density estimator for point cloud extracted from convex hull area at
276 each depth), and solidity (spline interpolation of solidity through every depth). These distributions
277 are then discretized into 10 bins for downstream analysis.

278 However, point clouds are made of scattered points without connection information. Volume and
279 length-related features cannot be directly measured. To be able to compute the volume dependent
280 features, we compute alpha shapes with a set of radii to form a few bounding volumes that envelop
281 the point cloud (Edelsbrunner et al., 1983). Intuitively, an alpha shape is formed by scooping out ice
282 cream with a sphere spoon without bumping into chocolate pieces (the points) and then straightening
283 the boundaries. The size of a spoon is a parameter denoted as alpha. We measure these alpha shape
284 volumes with three different scales (alpha = 0.5, 1, and 2) which indirectly describe the root volume
285 (Fig 6). We calculate the solidity using the ratio between alpha shape volume at alpha = 2 and convex
286 hull volume. To be able to compute the length dependent features, point cloud is skeletonized into a
287 network system using an algorithm based on a Laplacian contraction method (Cao et al., 2010),
288 which was conducted in Matlab R2017a. Then we can calculate length dependent features such as the
289 total root length.

290 **2.7 Root system 3D biomass**

291 Biomass measurements are taken by utilizing a grid system. Each layer of the mesocosm, starting
292 from the bottom, may contain biomass and is weighed. This process starts by identifying the location
293 of the sample in the coordinates created by the fishing line structure. Mass is weighed by cutting the
294 roots at each layer and recording the weight within each box. After completing each layer, the crown
295 of the root is then removed, labeled, and stored for further analysis.

296

297 **3 Results**

298 **3.1 Species and genotype modeling facilitated by the mesocosm systems**

299 We successfully grew and modeled the entire root system architectures of mature (after flower
300 formation) maize (PHZ51), sorghum (BTX623), and switchgrass (WBC3, VS16) (Fig 7;
301 Supplemental Videos 1-4, Table 1) in Turface MVP (Profile Products LLC., Buffalo Grove, Ill) using
302 our 3D Root Mesocosms. Variation in the root systems of these species is evident both by eye and
303 through the analysis of the subsequent point clouds developed through photogrammetry. The bulk of
304 our studies focused on two key switchgrass varieties that have adapted to different natural
305 environments: upland (VS16) and lowland (WBC3) switchgrass (Milano et al., 2016). The distinct
306 root system architectures of these genotypes are apparent (Fig 8A, D, G). While the upland VS16
307 genotype is smaller, it shows much less horizontal growth compared to the lowland WBC3,
308 apparently prioritizing carbon allocation to deeper rooting under our experimental conditions.
309 Furthermore, VS16 shows more vigorous lateral root growth relative to the total root system size and
310 has a higher root to shoot ratio, responses believed to aid in capturing as much water as possible from
311 the local environment. Conversely, WBC3 shows a much wider horizontal spread of water
312 transporting axile roots coupled with less investment into water absorbing lateral roots, a pattern
313 expected in plants adapted to environments with ample water availability (Weaver, 1926).

Root System Architecture and Environmental Flux Analysis in Mature Crops using 3D Root Mesocosms

314

315 **3.2 RSA model accuracy confirmed by 3D biomass ground truth**

316 To ensure that the point clouds derived via photogrammetry are accurate to the actual RSA, a direct
317 comparison to biomass in 3D space was necessary. Using the location of the internal mesocosm
318 fishing line scaffold coordinates the root systems were dissected both physically and computationally
319 (Fig 9, Fig S4). Using the 810 individual subunits formed by the scaffold the point cloud and biomass
320 can be compared at a 10 cm x 10 cm x 15.25 cm resolution. Biomass ground truth measurements
321 align well with *in silico* generated cubes of the point clouds that occupy the same space (Fig S5).
322 Scaling the values of each coordinate section to the entirety of the growth space, the biomass and
323 voxel amount, can be directly compared.

324 Beyond acting as a ground truth for point clouds, the biomass measurements obtained give an
325 unprecedented sampling of entire root systems of full-grown crop plants largely preserved in their
326 natural configurations. Out preliminary experiments show that differences can be observed between
327 switchgrass genotypes, as well as in response to water stress (Fig 10). When grown under well-
328 watered conditions both WBC3 and VS16 root systems displayed a similar profile of biomass
329 allocation with depth, with the majority of biomass allocated in the upper profile and less allocated to
330 each subsequent depth. In contrast, when WBC3 plants were grown under water stressed conditions
331 the biomass allocation was modified and near-even amounts of root tissues were distributed at all
332 depths down to 3 ft (91.44cm).

333

334 **3.3 Mesocosms as a platform for water deficit experiments**

335 The ready control and measurement of various environmental conditions in the 3D root mesocosms
336 was demonstrated using a TEROS21 sensor array (Fig 2) to investigate the 3D root system
337 phenotypic response of WBC-type switchgrass to physiologically-defined water stress. The high
338 spatial and temporal resolution of our imputed sensor data (Fig S1) facilitated 4D monitoring of
339 water fluxes from which we made delicate adjustments of irrigation to impose two levels of water
340 availability: a well-watered treatment with a constant matric potential of -0.01 MPa and a water stress
341 treatment with the average stress levels of approximately -2.5 MPa.

342 Continuously monitoring the matric potential revealed the real-time dynamics of water deficit
343 throughout the duration of plant growth, including diurnal patterns of wetting and drying tied to daily
344 transpiration (Fig S6). The TEROS21 system simultaneously collects temperature data which can be
345 analyzed in conjunction at the same resolution (Fig S6). We note the temperature gradient in our
346 system mimics field soils to an extent, insofar as temperature decreases with depth.

347 Several hallmarks of traditional responses to water deficit were seen in WBC3 when grown under the
348 moderate-to-severe level of stress (-2.5 MPa), including a major reduction in root system volume and
349 convex hull, but with maintenance of overall root system depth (Fig 8, B, E; Supplemental Video 5),
350 leading to a significant shift of the root to shoot ratio (Table 1). The tradeoff to maintaining depth
351 with a smaller root mass is a reduced global solidity, which quantifies the thoroughness of soil
352 exploration in the rooting zone (defined by the convex hull volume). Analysis of root system traits
353 across the depth profile revealed the biomass and convex hull area of water-stressed WBC3 was
354 larger than well-watered below ~12 inches (~30cm), revealing allocation of more biomass (point
355 number) to root proliferation at depth (Fig 8G). However, in the upper profile WBC3 displayed more

Root System Architecture and Environmental Flux Analysis in Mature Crops using 3D Root Mesocosms

356 biomass and a larger convex hull under well-watered conditions compared to water stressed, with
357 ~71% of the total root mass in the top ~12 inches.

358

359 **3.4 Assessing effects of growth media on RSA and the root zone environment**

360 To study the effects of growth media on RSA and environmental parameters, we explored the
361 incorporation of standard greenhouse potting mix (Berger BM7, Berger Saint-Modeste, QC) into the
362 system under well-watered conditions (Fig 8C, F, G). When grown in a mix of 3:1 potting mix to
363 surface WBC3 plants appeared to have longer and less branched lateral roots than when grown in
364 pure surface (Fig S7). We suspect this change is a response to the particle size of the potting mix,
365 which is much smaller than the average surface particle, and has a greater hydraulic conductivity.
366 Roots growing through smaller potting mix particles require less lateral branching to access growth
367 media bound water as there would be significantly more root-to-particle contact points along the root
368 compared to growth in the surface. The VS16 plants developed very small root systems under the
369 mixed media compared to the surface, as well as reduced root to shoot ratios, perhaps reflecting that
370 they are not adapted to grow in an extremely wet environment (Fig 8 C, F, G; Table 1).

371 We used a different facet of our 3D sensor array to monitor dynamic CO₂ respiration from soils (Fig
372 11). Both root and microbial respiration are major drivers of subsoil CO₂ production, and rhizosphere
373 processes such as microbial consumption of root exudates and soil organic matter link these pools.
374 Surface is a calcined clay product and contains little or no organic matter (OM) (Beddes et al., 2013;
375 Beddes and Kratsch, 2009; Calonje et al., 2010), whereas greenhouse potting mix typically has a very
376 high OM content (in our case Berger BM7 is ~79%). In surface at early time points, VS16 and WBC3
377 switchgrass CO₂ profiles are very similar, although VS16 is set higher (Fig 11C, D). Over time
378 (around week 8) CO₂ levels at all three measured depths begin to rise, presumably as a result of rapid
379 root proliferation. However, WBC quickly rises several-fold at the lowest depth (4.5 feet), consistent
380 with differences in its eventual root system size (Fig 8A).

381 Interestingly this same relationship is not seen in mesocosms filled with a 3:1 potting media: surface
382 mix. Under these conditions the CO₂ levels were several orders of magnitude higher compared to
383 surface filled mesocosms and the levels remained constant or showed a slight decline throughout the
384 growth term (Fig 11A, B; Fig S8). The significantly higher CO₂ levels and their stability at the
385 sample locations at the middle and higher elevations suggest that a combination of the organic
386 components and microbial population of the potting mix play a more significant role than direct root
387 respiration. Yet, WBC3 mesocosms showed elevated CO₂ levels in the lowest growth media profile
388 compared to VS16 mesocosms, an area where VS16 root systems did not occupy (Fig 8A). This
389 result suggests that local root activity at depth in WBC samples may be driving increased microbial
390 activity via rhizosphere priming (Kuzyakov, 2002) (Fig 11A, B).

391

392 **3.5 Combined analysis of RSA models and 3D environmental data**

393 Aligning the photogrammetry point clouds with the time course 3D environmental data fluxes
394 provides the opportunity to make post hoc hypotheses on how the environment shaped the mature
395 RSA. Alterations in matric potential and temperature in the growth media along the path of root
396 development can give insight into the conditions that resulted in the RSA, and changes in sub soil

Root System Architecture and Environmental Flux Analysis in Mature Crops using 3D Root Mesocosms

397 CO₂ are correlated with the presence of root respiration (Fig 12; Supplemental Videos 6, 7). This
398 type of analysis can be used to make observations to provide training data to a model in an effort to
399 estimate root location and activity based on localized environmental fluxes. Monitoring root system
400 width and depth changes over time via proxy measurements is a promising idea that could provide an
401 avenue to non-destructive root system shape measurements and time course analysis of root system
402 development.

403 Further, direct comparisons of RSA to environmental conditions can be achieved at the cuboid level,
404 and we have seen interesting preliminary data demonstrating a root system's capacity to affect its
405 surroundings. 3D interpolated environmental data was partitioned into 9x9x10" cuboids similar to the
406 biomass measurement described in 3.2. We labeled the cuboid that contains root as 'root cuboid' and
407 the cuboid that does not contain root as 'non-root cuboid'. For every layer, we calculated the average
408 matric potential among root cuboid and the average matric potential among non-root cuboid. The
409 data shows that under a well-watered condition, the matric potential of root and non-root cuboids are
410 almost identical. However, under water stressed conditions, the root cuboids are consistently wetter
411 at every layer, with the effect being more obvious at top layers that have more water availability than
412 the bottom layers (Fig 13). We suspect this result indicates that active root uptake is drawing water
413 into the root occupied regions from those without, and hints at the potential to infer a coarse 3D root
414 system architecture over time from embedded sensor data.

415

416 **4 Discussion**

417 **4.1 A 3D Root Mesocosm System for Integrated Environmental Sensing and Root** 418 **Phenotyping**

419 The concept of mesocosms in plant biology have been used widely to refer to a variety of
420 experimental systems. From assessing the effects of invasive European earthworms on North
421 American tree growth (Hale et al., 2008), to the reduction in soil-mercury emission due to soil
422 shading by vegetation (Gustin et al., 2004), or the effects of sediment nutrition and light resources on
423 seagrass growth and development (Short, 1987; Short et al., 1995), mesocosms are a useful
424 intermediate between the laboratory and the field (Odum, 1984).

425 Root mesocosms, typically large horticultural pots or long narrow pipes from which the entire root
426 system can be extracted, have proven useful in understanding RSA and root function of several
427 agricultural species. For examples: it was reported that a lower number of crown roots in maize can
428 be beneficial for nitrogen acquisition in poor nitrogen soils (Saengwilai et al., 2014), a moderate
429 progressive drought could lead to RSA adaptations in various rice cultivars that improve performance
430 under reduced water management practices (Hazman and Brown, 2018), some Green Revolution
431 wheat progenitors have smaller root systems than older landraces (Waines and Ehdaie, 2007), and
432 Chilean red clover cultivars with certain RSA traits, such as high crown root diameter and low
433 branching index, correlate with superior persistence (Inostroza et al., 2020). However, in these
434 studies the root systems were physically constrained during growth, leading to, at minimum,
435 compromised estimates of root length densities and other metrics across the depth profile. To our
436 knowledge, we report here the first system to grow large crop plants to maturity and recover
437 unconstrained, intact root systems in their nearly-natural configuration. With the accompanying
438 imaging and analysis plus sensor data, we have developed a new, flexible, paradigm for
439 comprehensive subterranean analysis of root and rhizosphere biology.

Root System Architecture and Environmental Flux Analysis in Mature Crops using 3D Root Mesocosms

440 4.2 Next Generation Mesocosms

441 New versions of the mesocosm are being developed to expand the scope and versatility of the
442 technology. A large-scale version, measuring appx. 3 m wide x 6 m long x 2 m tall is being
443 developed to more closely replicate field dynamics. In this “common-garden” or “plot-level” system,
444 rows of plants can be placed across several frames to begin to understand multi plant dynamics. We
445 have also added a robotic imaging system for high-throughput above-ground plant imaging. Another
446 version is modular, with subsystems for analyzing plants with smaller root systems such as rice,
447 wheat, and covercrops. Several can also be connected together to create fewer, but larger units as
448 experimental needs change. These systems will accommodate a wider variety of sensors and allow
449 access to different depths through a series of ports that allow root and rhizosphere sampling in situ.
450 An important goal is to improve the realism of the system, and although we used artificial growth
451 media in this study, in principle, any reconstituted soil or soil-substrate can be used. Considerations
452 include the weight of the system and the ease and efficacy of recovering root systems intact.

453 4.3 The importance of capturing entire 3D root system architectures grown nearly 454 unconstrained

455 Photogrammetry has many uses in plant biology and is a field of rapidly evolving interest (Iglhaut et
456 al., 2019). Drone based imagery has been widely adopted as a tool to evaluate forest coverage, health
457 and activity (Goodbody et al., 2019; Iglhaut et al., 2019; Miller et al., 2000; Mlambo et al., 2017).
458 Similarly, terrestrial based projects such as assessments of the shape of individual trees (Bauwens et
459 al., 2017; Gatziolis et al., 2015; Marín-Buzón et al., 2020; Wang et al., 2004) or various fruits
460 (Feldmann and Tabb, 2022; Gené-Mola et al., 2021; Ni et al., 2021) have also become more
461 common. A recent study has shown the power of optical reconstructions for 3D analysis of root
462 crowns (Liu et al., 2021). However, to the authors' knowledge, the 3D Root Mesocosms are the first
463 system to generate 3D reconstructions of entire full grown crop root systems in nearly natural
464 configurations, from any method.

465 Although the imaging of samples using photogrammetry is a low-cost process that does not require
466 significant infrastructure, there are several challenges that still remain. Unlike other tomographic
467 techniques, such as X-ray CT (Shao et al., 2021), photogrammetry does not resolve internal
468 structures of the sample as the 2D images are only capturing surface features within line of sight of
469 the 2D-photographs. This means that dense root crowns or areas of thick matted lateral roots are not
470 resolvable. Thus, we are considering the potential to complement the photogrammetry derived point
471 cloud with X-ray CT derived root crown reconstructions (Shao et al., 2021; Zeng et al., 2021).
472 Additionally, photogrammetry at such a large scale can require significant computation power,
473 dedicated software, and can currently take on the order of days to process each sample. Even
474 considering these limitations, photogrammetry still represents a powerful tool to generate 3D models
475 of root architecture that is flexible to image a wide array of samples, and is comparatively low-cost in
476 relation to other tomographic methods.

477 The development of entire 3D root system models based on actual (non-computer-generated) plants
478 also provides an opportunity to assess the amount of error inherent to a range of commonly utilized
479 field-based root phenotyping methods such as soil cores, minirhizotrons, and shovelomics, which
480 seek to estimate entire root systems from partial sampling (Pagès and Glyn Bengough, 1997;
481 Trachsel et al., 2011; Wu et al., 2018). One idea is to generate *in silico* soil cores or minirhizotron
482 images from the point clouds. This method could provide a sensitivity analysis for empirical

Root System Architecture and Environmental Flux Analysis in Mature Crops using 3D Root Mesocosms

483 sampling strategies using actual, rather than virtual (Burridge et al., 2020; Morandage et al., 2019),
484 groundtruths. Such information could also be used as a valuable resource for improving root
485 structure-function simulations (Kalogiros et al., 2016; Postma et al., 2017; Schnepf et al., 2018), or
486 for the development of artificial intelligence approaches to complement missing data (Falk et al.,
487 2020; Gaggion et al., 2021; Ruiz-Munoz et al., 2020).

488 **4.4 Conclusion**

489 The field of root system architecture phenotyping has advanced dramatically over the last few
490 decades, from simple measurements taken with a ruler to the development of interactive virtual
491 reality platforms. While the core complications of root phenotypic and functional analysis
492 remain, advances along several avenues have allowed researchers to begin to analyze and visualize
493 the subterranean dynamic complexities of root systems. We believe that, currently, coupling
494 mesocosms and photogrammetry is a powerful way to assess the 3D structure of full grown,
495 unconstrained root systems in their natural configurations. The methods detailed here are easily
496 adapted to fit any size of plant and can be scaled appropriately to study concepts such as plant to
497 plant root system interactions or planting density effects on RSA in a relatively inexpensive and easy
498 to build manner. Further, the ease of incorporating various sensors or sampling schemes at the
499 desired locations in the subterranean profile provides an unprecedented freedom to target specific
500 areas of the root system to observe architectural traits and root function.

501

502 **5 Conflict of Interest**

503 The authors declare that the research was conducted in the absence of any commercial or financial
504 relationships that could be construed as a potential conflict of interest.

505

506 **6 Author Contributions**

507 Dr. Tyler Dowd developed the initial mesocosm design and lead in their physical construction,
508 established the photogrammetry workflow to develop 3D model from the root systems, lead the
509 selection and implementations of environmental sensors, conducted the bulk of photogrammetry and
510 subsequent point cloud cleaning, development the measurement of biomass collection to ground truth
511 the point clouds, conducted all subterranean CO₂ measurements, and was the primary author of
512 writing the manuscript.

513 Dr. Mao Li developed the semi-automated cleaning and segmentation of the root system point
514 clouds, the methods of root trait extraction from the isolated root system point clouds, the
515 visualization and modeling of 3D sensor data, the complementation of the various sensor data with
516 the root system point clouds, and assisted in the writing of the manuscript

517 Dr. G. Cody Bagnall developed subsequent mesocosm designs, led the construction of mesocosms
518 after Generation 1, developed the blueprint of the construction figures, and assisted in the writing of
519 the manuscript.

Root System Architecture and Environmental Flux Analysis in Mature Crops using 3D Root Mesocosms

520 Andrea Johnston assisted in the imaging of mesocosm root systems, the cleaning of resultant point
521 clouds, the biomass collection to provide ground truth measurements, and assisted in the writing of
522 the manuscript.

523 Dr. Christopher Topp conceived the work, oversaw and coordinated the research, and contributed to
524 writing of the manuscript.

525

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529

530 **8 Abbreviations**

531 Magnetic Resonance Imaging (MRI), Root System Architecture (RSA), X-ray computed tomography
532 (X-ray CT).

533

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539 Alina Zare for their assistance with point system skeletonization, and Dr. Samuel McInturf for the
540 development of an R script to process Picarro Data.

541

542 **10 Supplementary Material**

543 Supplemental Figure 1: Interpolation of 3-dimensional environmental sensor data.

544 Supplemental Figure 2: Time course of shoot morphological responses of switchgrass in different
545 growth media.

546 Supplemental Figure 3: Manual post-process cleaning of RSA point clouds.

547 Supplemental Figure 4: Comparison of the biomass and point number located in each cuboid
548 throughout the mesocosm growth zone.

549 Supplemental Figure 5: Dissection of mesocosm grown root system for biomass measurements

550 Supplemental Figure 6: Diurnal environmental fluxes in mesocosms across nine days.

551 Supplemental Figure 7: Growth media effects on lateral root architecture

Root System Architecture and Environmental Flux Analysis in Mature Crops using 3D Root Mesocosms

- 552 Supplemental Figure 8: Subterranean CO₂ flux monitoring in mesocosms.
- 553 Supplemental Video 1: A 360-degree rotation of a photogrammetry generated point cloud of a
554 Sorghum (BTX623) root system grown in turface under well-watered conditions.
- 555 Supplemental Video 2: A 360-degree rotation of a photogrammetry generated point cloud of a Maize
556 (PHZ51) root system grown in turface under well-watered conditions.
- 557 Supplemental Video 3: A 360-degree rotation of a photogrammetry generated point cloud of a
558 Switchgrass (WBC3) root system grown in turface under well-watered conditions.
- 559 Supplemental Video 4: A close up 360-degree rotation of a photogrammetry generated point cloud of
560 a Switchgrass (WBC3) root crown grown in turface under well-watered conditions.
- 561 Supplemental Video 5: A 360-degree rotation of a photogrammetry generated point cloud of a
562 Switchgrass (WBC3) root system grown in turface under well-stressed conditions.
- 563 Supplemental Video 6: A photogrammetry generated point cloud of a Switchgrass (WBC3) root
564 system grown in turface under well-stressed conditions coaligned with diurnal matric potential flux
565 data over 7 days.
- 566 Supplemental Video 7: A photogrammetry generated point cloud of a Switchgrass (WBC3) root
567 system grown in turface under well-stressed conditions coaligned with diurnal temperature flux data
568 over 7 days.

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Root System Architecture and Environmental Flux Analysis in Mature Crops using 3D Root Mesocosms

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Table 1: Root-to-shoot ratios of various species and treatment combinations grown in 3D mesocosms
Biomass data of dried shoot and roots weights, and the corresponding root-to-shoot ratios, for switchgrass (WBC3, VS16), sorghum, and maize grown in turf or mixed media under well-watered or water-stressed conditions. Data are means \pm standard error, n= 3 for all treatments except Sorghum WS Turf (n=2) and Maize WW Turf (n=6).

	Shoot Weight (g)	Root Weight (g)	Root: Shoot Ratio
WBC3 WW Mixed Media	265.3 \pm 37.8	126.5 \pm 26.9	0.48
WBC3 WW Turf	271.1 \pm 79.5	144.9 \pm 41.7	0.53
WBC3 WS Turf	44.5 \pm 14.6	52.9 \pm 14.3	1.19
VS16 WW Mixed Media	36.2 \pm 16.4	22.0 \pm 1.6	0.61
VS16 WW Turf	114.8 \pm 23.3	98.6 \pm 8.6	0.86
Sorghum WW Turf	257.6 \pm 28.1	77.6 \pm 2.0	0.30
Sorghum WS Turf	98.7	80.3	0.81
Maize WW Turf	75.9 \pm 12.2	62.1 \pm 8.4	0.87

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Root System Architecture and Environmental Flux Analysis in Mature Crops using 3D Root Mesocosms

747 Figure Legends:

748 Figure 1: Major structural components of the mesocosm system. The mesocosm base is constructed
749 from pressure treated lumber and designed for easy movement by a pallet jack (A). Directly above
750 the base is a drainage box equipped with a drain spout to facilitate flow-through irrigation and allow
751 sample collection (B). The internal component of the mesocosm is a scaffold system constructed of
752 0.5 inch PVC and fishing line (C). The exterior mesocosm walls are composed of lumber and held
753 together with galvanized bolts (D). Between the internal frame and external lumber are thin
754 boundaries of masonite that form smooth interior surfaces and a tarp to hold water in. The boards on
755 the outside of the mesocosm can be attached and removed for easy access to the interior scaffold after
756 roots have been grown.

757 Figure 2: Interior sensor layout of the mesocosm. The interior PVC and fishing line scaffold create a
758 coordinate system that can be used for sensor placement and data interpolation. Various
759 environmental sensors (black spheres) were placed in a grid formation at 3 elevations throughout the
760 mesocosm growth profile, 1.25 ft, 2.5 ft, and 4.25 ft deep. At the two upper elevations 5 sensors are
761 laid out in a cross pattern while on the lowest level there are 4 with the center sensor absent. The
762 lower right panel shows a photograph of both TEROS21 matric potential/ temperature sensors as well
763 as air intake tubes for a Picarro gas analyzer.

764 Figure 3: Mesocosm harvest method. Mesocosms supporting the growth of full size WBC3 and VS16
765 switchgrass genotypes (A). At harvest the shoots are cut a few centimeters above the soil profile (B).
766 Shoots are bundled and dried for biomass measurements to accompany other shoot morphological
767 traits collected during growth (C; Fig S2). Harvest begins by removing the uppermost exterior 2x6s,
768 removing the masonite and pulling back the tarp to expose the top of the growth profile for
769 excavation (D). Throughout sensors will be carefully extracted from the root system so as not to
770 disturb the architecture (E). Harvest continues until all root tips are exposed from the growth media
771 (F). Following complete excavation, the root system can be relocated in the PVC and fishing line
772 scaffold and stored for future analysis (G).

773 Figure 4: Photogrammetry studio and imaging process. Photogrammetry requires a dedicated space
774 with many unique identification markers and strong uniform illumination (A). Plants samples are
775 placed centrally in the studio to minimize shadows. Images are taken surrounding the subject (small
776 rectangles are locations of individual camera locations) at several elevations to provide data to form
777 the environment (C). Close up images are taken surrounding the root system with very high overlap
778 to produce maximum system detail (D).

779 Figure 5: Semi-automated segmentation of the root system point cloud. Point cloud of the root
780 system with the scaffold is aligned to a predefined reference model by performing linear transform on
781 manually picked control points to the target points (A). Point cloud of the scaffold is removed based
782 on the position and color information (B). Blue noise on the root is then removed using a threshold
783 method (C). A post manual processing is conducted to further clean the root system point cloud (D).
784 Point cloud is skeletonized into a network system using an algorithm based on a Laplacian

Root System Architecture and Environmental Flux Analysis in Mature Crops using 3D Root Mesocosms

785 contraction method (E).

786 Figure 6: Trait extraction via alpha shape analysis. An alpha shape is a kind of shape that envelopes
787 the point cloud (A). Intuitively, it is formed by scooping out ice cream with a sphere spoon without
788 bumping into chocolate pieces (the points) and then straightening the boundaries. The volumes of
789 alpha shape can be calculated for different parameters such as $\alpha=0.5$ (B) and $\alpha=2$ (C).

790 Figure 7: RSA of 3 different mesocosm grown species (left). Representative point clouds for
791 sorghum, maize, and switchgrass species. Orange dotted line denotes the approximate growth media
792 level during growth. A radar plot detailing the analysis of 7 different root shape traits from the point
793 clouds. Data shown are mean \pm standard error in shaded regions. Sorghum and switchgrass $n=3$,
794 maize $n=2$.

795 Figure 8: RSA traits of switchgrass affected by genetics and environmental conditions.
796 Representative point clouds and extracted root traits of various G x E experimental conditions
797 examinable via mesocosms. Genotypic comparison of WBC3 (orange) and VS16 (blue) when grown
798 in well-watered surface (A, D). RSA response of WBC3 to well-watered (orange) and water stressed
799 (yellow) surface conditions (B, E). RSA response of VS16 when grown in well-watered surface (blue)
800 or a 3:1 potting mix to surface blend (green). Radar plots of data have all been standardized to allow
801 comparison across treatments and traits, data shown are mean \pm standard error. Point number
802 (biomass proxy), convex hull, and solidity trait values (G) are presented for the entire depth of the
803 growth media profile (WBC3 WW surface, orange; VS16 WW surface, blue; WBC3 WS surface,
804 yellow; VS16 WW mixed media, green). Values for solidity were transformed by $\log(x*10000)$ for
805 data visualization.

806 Figure 9: Biomass confirmation of point cloud accuracy. Comparison of data obtained from a
807 switchgrass root system that had been physically and digitally dissected into the 180 independent
808 sections outlined by the internal PVC and fishing line scaffold. Each gray square is a top-down view
809 of a z-layer consisting of 9 x 9 cuboids. Data within each square represents the number of points, or
810 the fraction of biomass, found in a cuboid as a percentage of the entire root system. Physical
811 segmented biomass values correlate well with values of point number located in the same cuboid
812 coordinate position when assessed on a relative scale, $R^2 = .088$.

813 Figure 10: Biomass allocation by depth of switchgrass. Plot shows biomass measurements
814 throughout the growth media profile for VS16 well-watered (blue), WBC3 well-watered (orange),
815 and WBC3 water stressed (yellow). Data values are means \pm standard error.

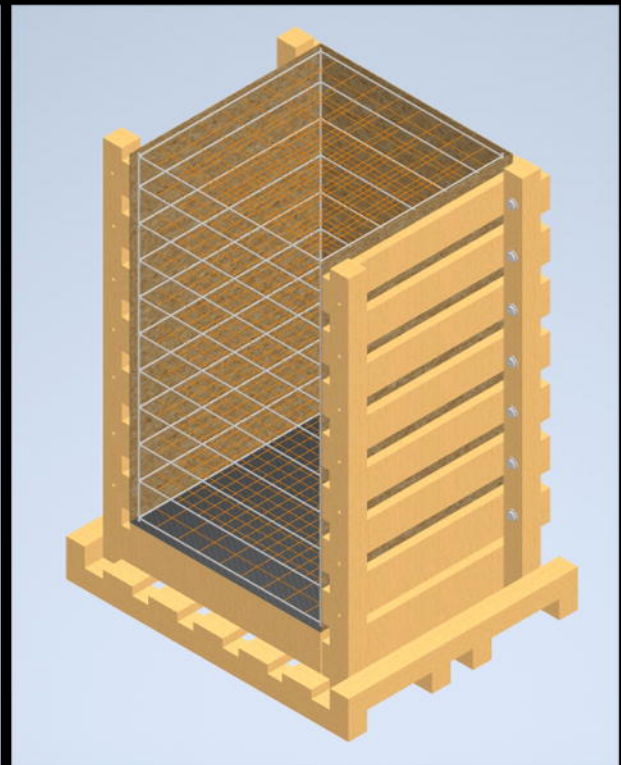
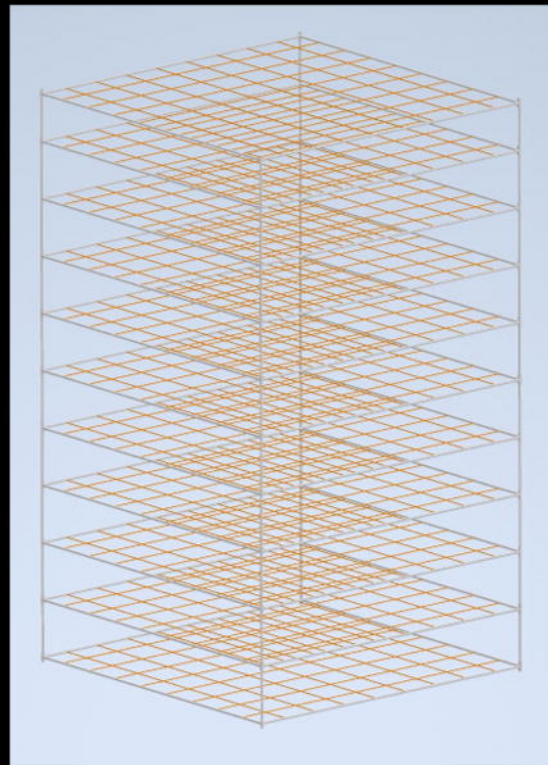
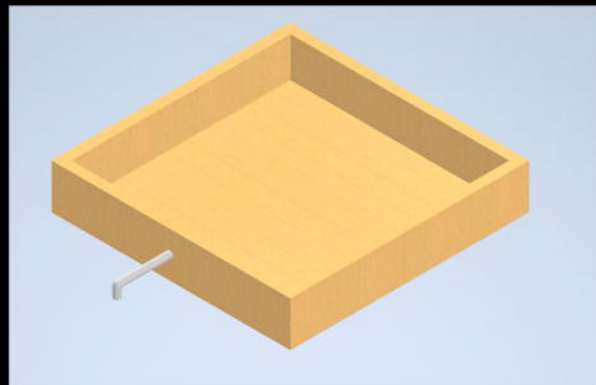
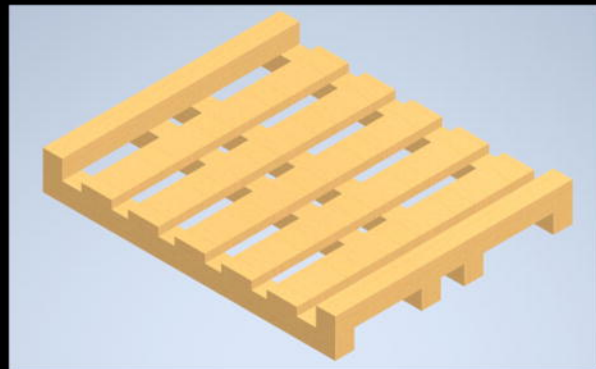
816 Figure 11: Subterranean CO₂ levels in the mesocosms are affected by which switchgrass genotype is
817 growing in what growth media. Graphs show CO₂ levels in mesocosms growing WBC3 (A, C) and
818 VS16 (B, D) grown in pure surface (C, D) and a 3:1 potting mix to surface blend (A, B).
819 Measurements were taken at three depths, 1.25 ft (gray), 2.75 ft (orange) and 4.25 ft (blue) below the
820 soil profile. Data values are means \pm standard error.

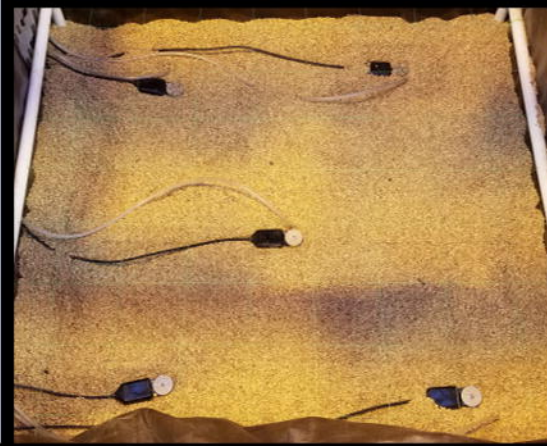
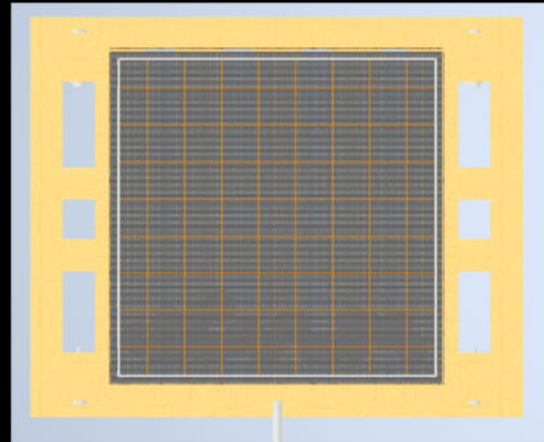
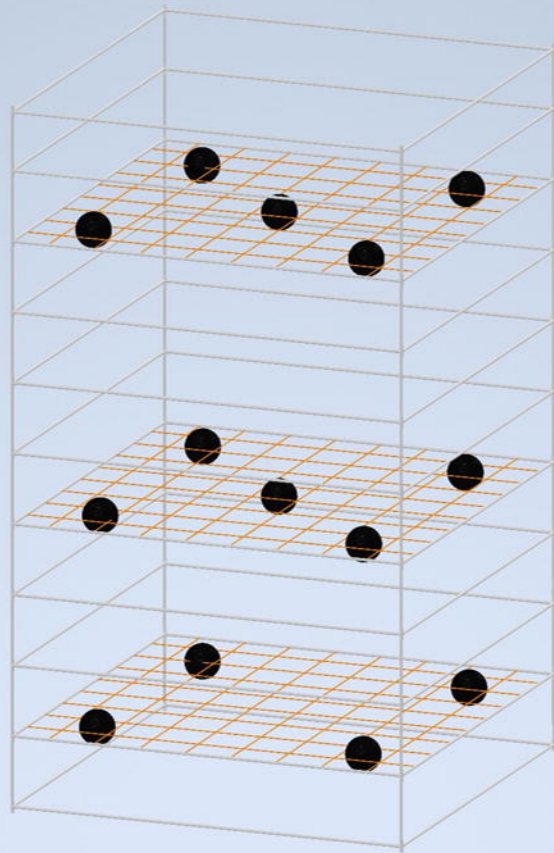
Root System Architecture and Environmental Flux Analysis in Mature Crops using 3D Root Mesocosms

821 Figure 12: Point cloud RSA models and environmental data synthesis. Data shows one switchgrass
822 point cloud with coaligned environmental 3D data for matric potential (A), temperature (B) and CO2
823 levels (C). All data are from the same time point collected between 11 am and 1 pm.

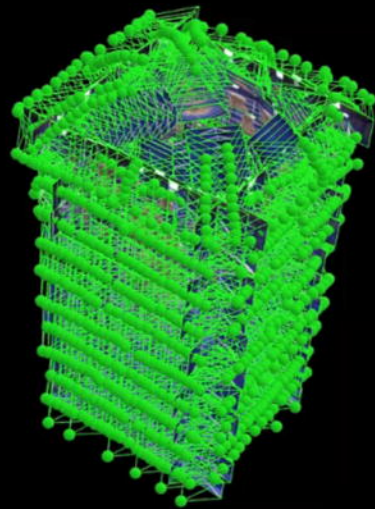
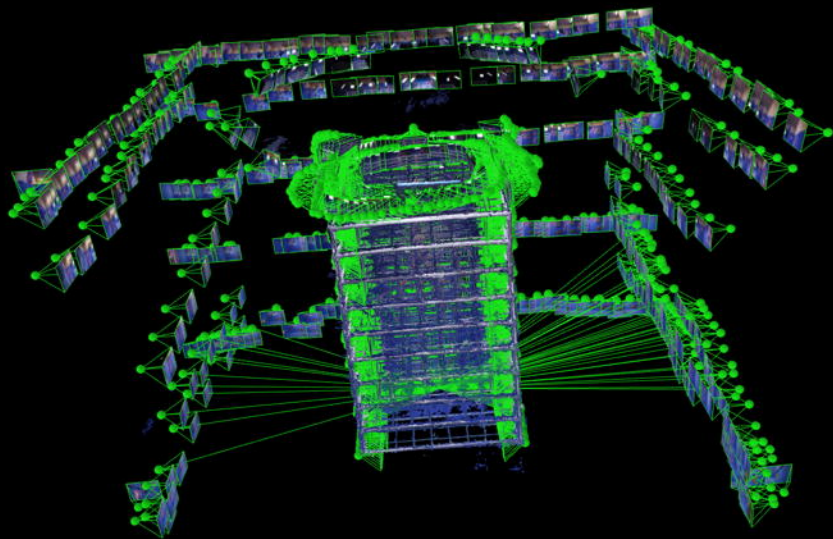
824 Figure 13: Bar plots of average matric potential for root cuboids and non-root cuboids at every layer
825 for a water stressed sample (A) and a well-watered sample (B). Values near zero (blue) represent
826 high water availability, while more negative values (red) denote a lower matric potential of the
827 growth media and lower water availability.

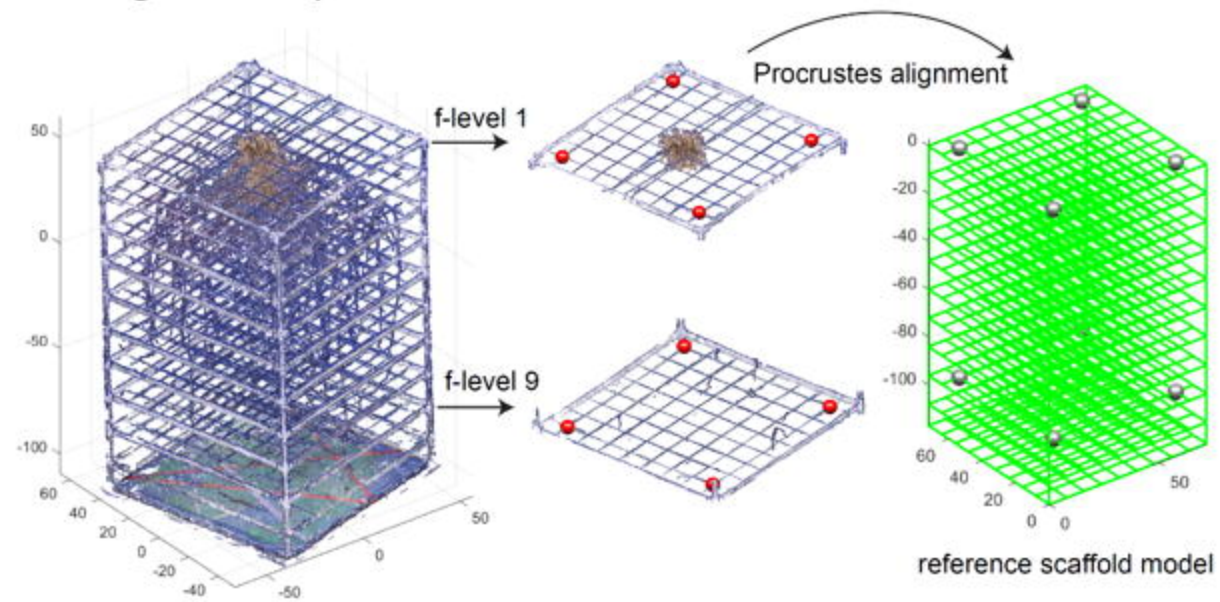
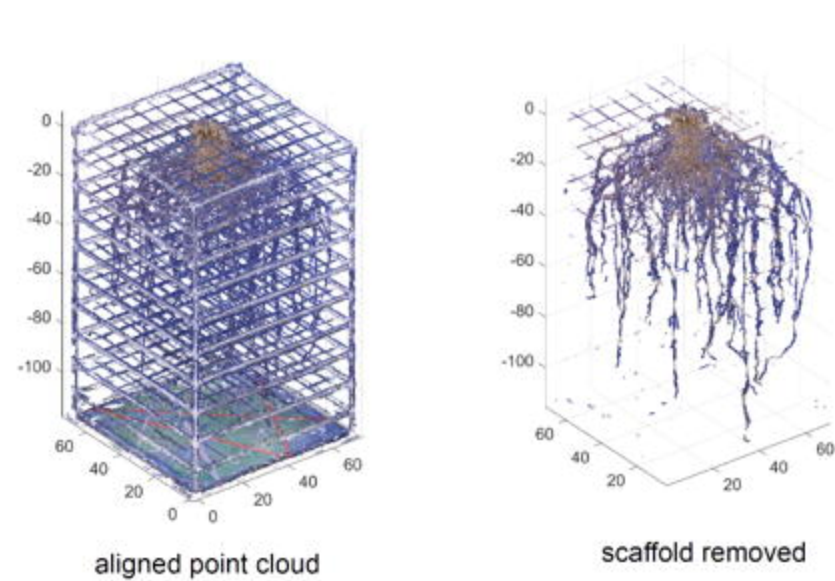
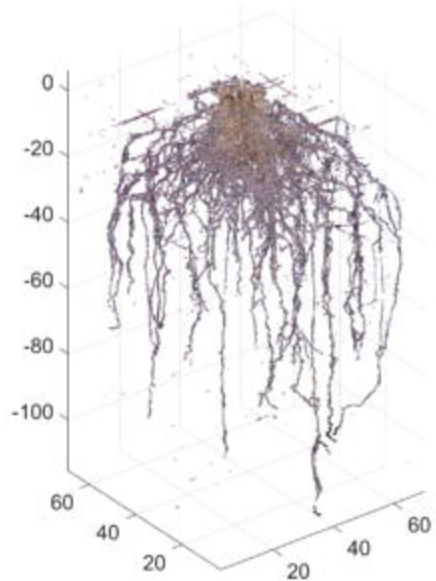
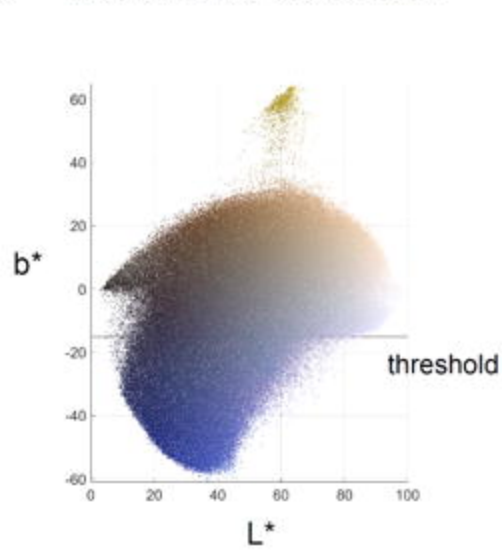
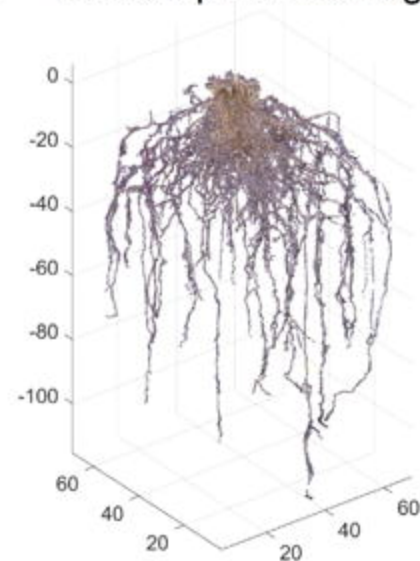
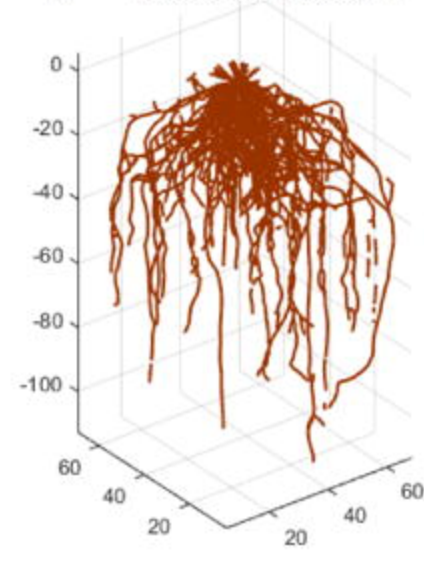
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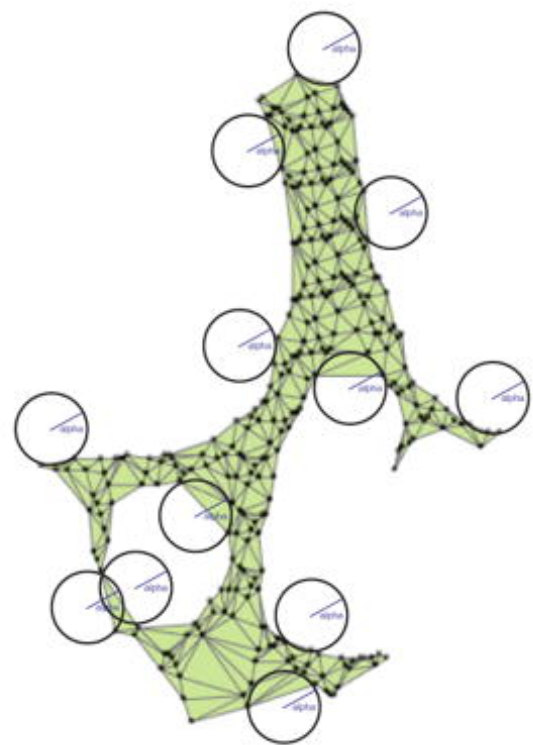




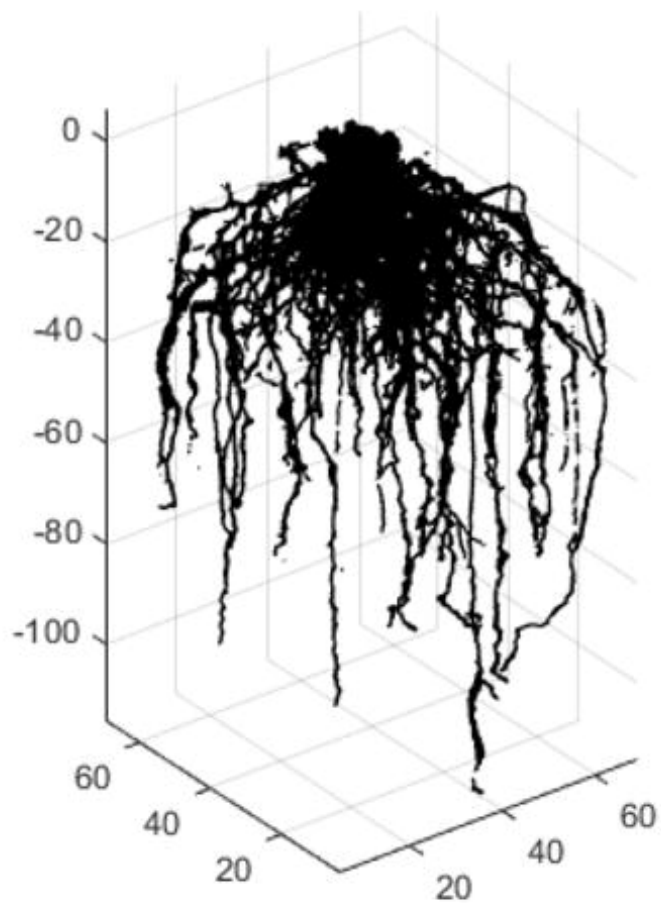


A Alignment of point cloud**B** Removal of scaffold**C** Removal of blue noise**D** Manual post cleaning**E** Skeletonization

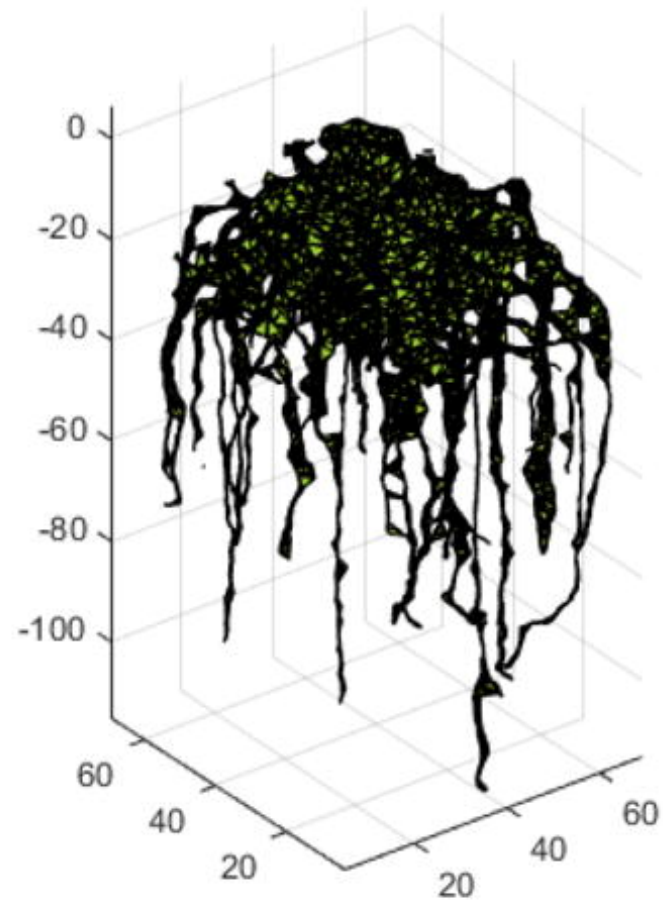
A  =alpha shape

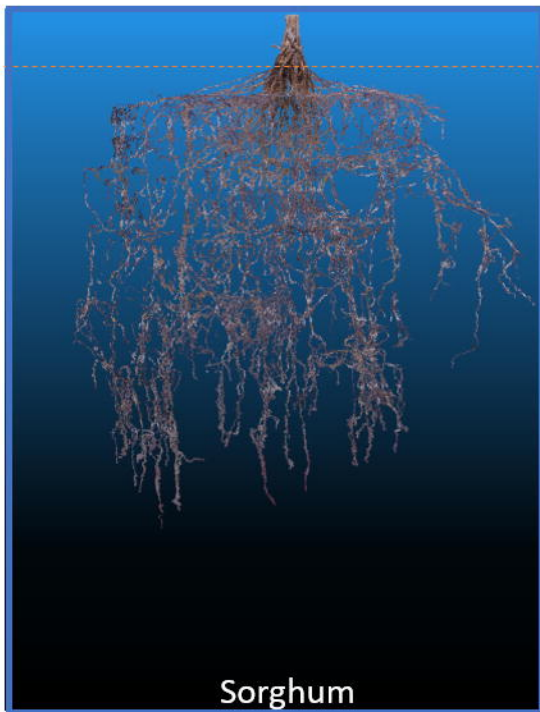
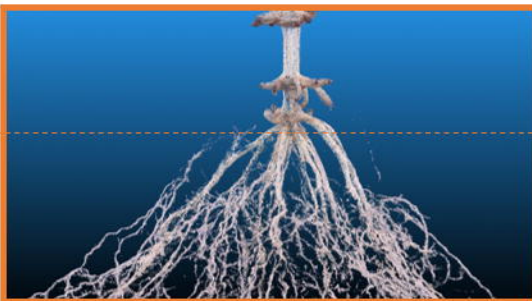
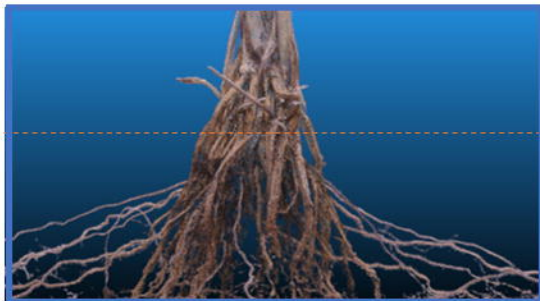


B alpha=0.5



C alpha=2





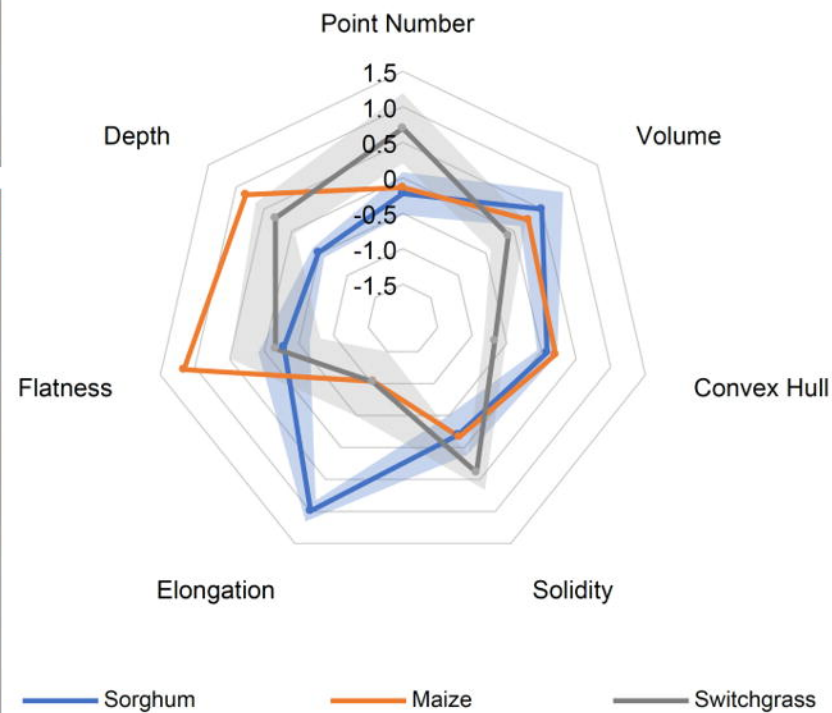
Sorghum

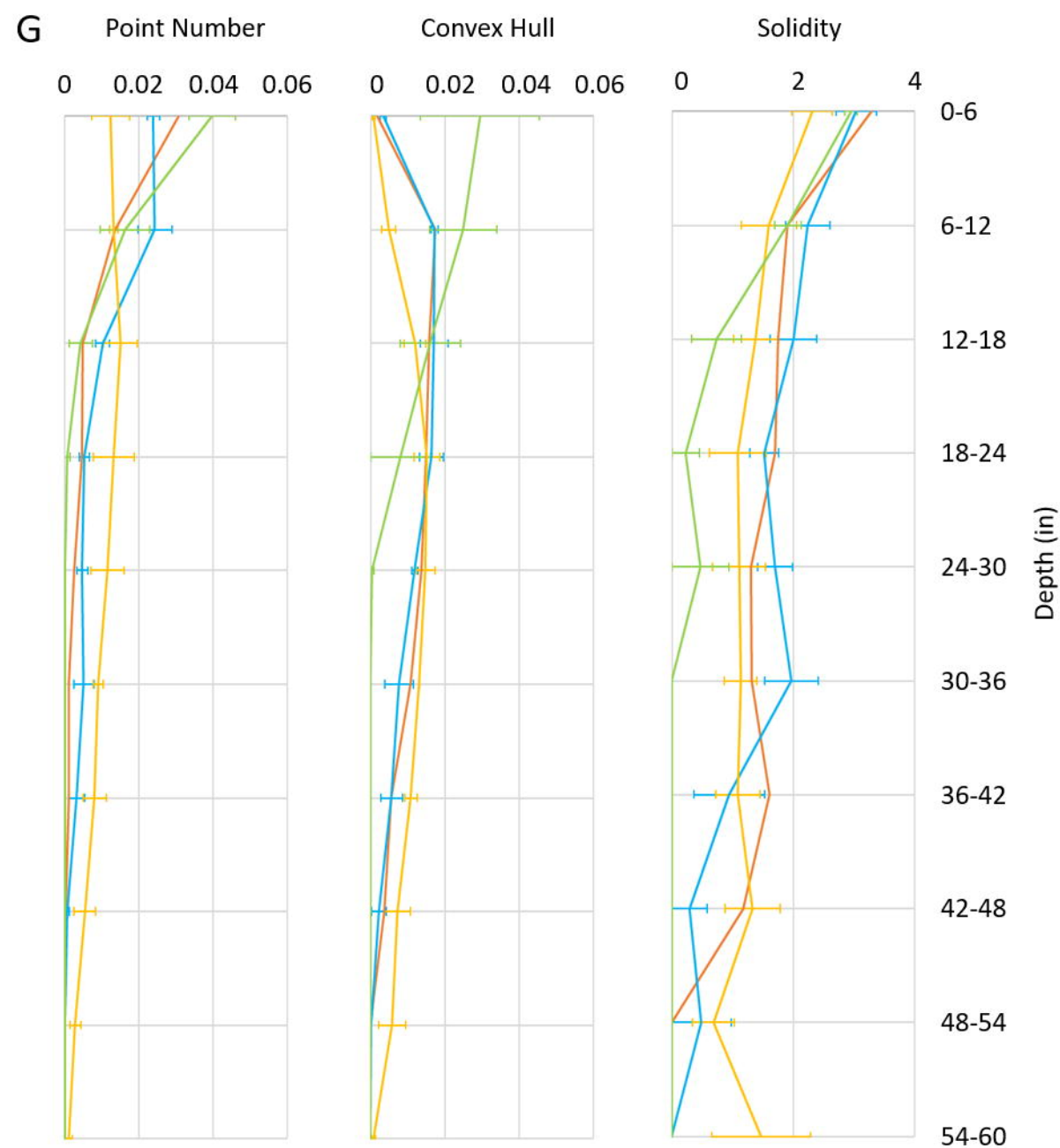
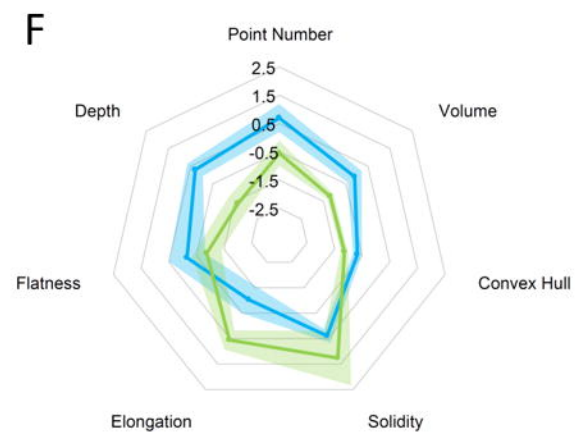
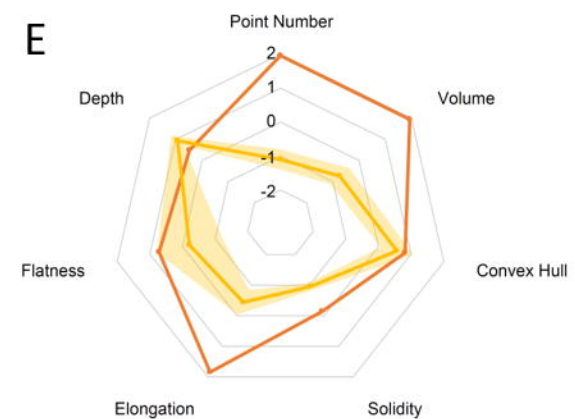
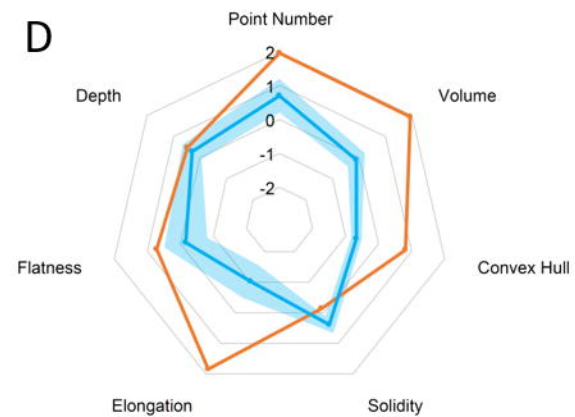
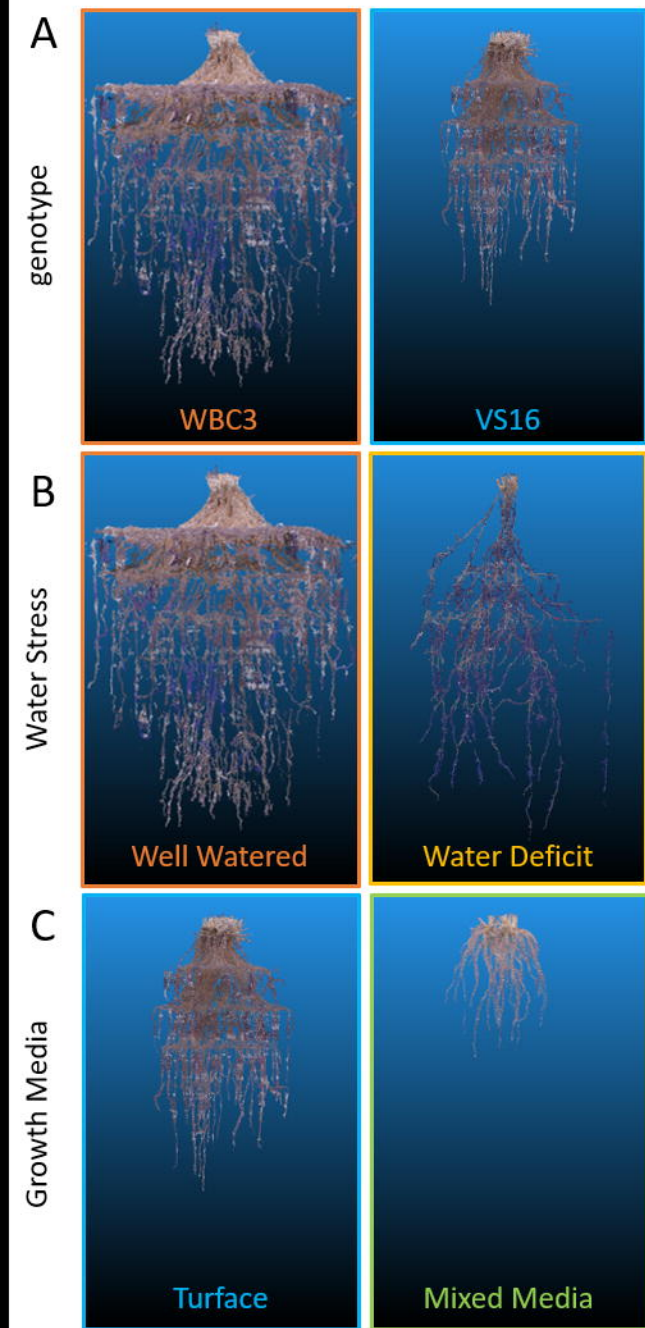


Maize



Switchgrass



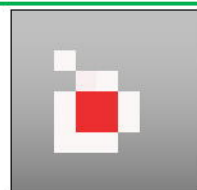


Depth (in)

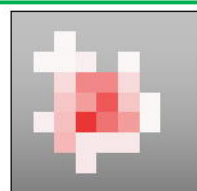
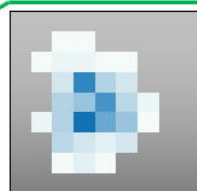
Points

Biomass

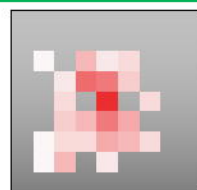
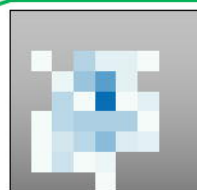
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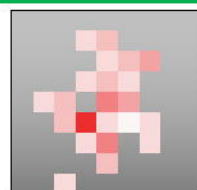
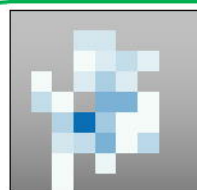
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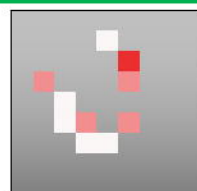
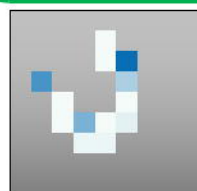
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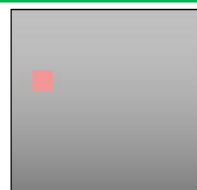
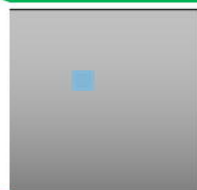
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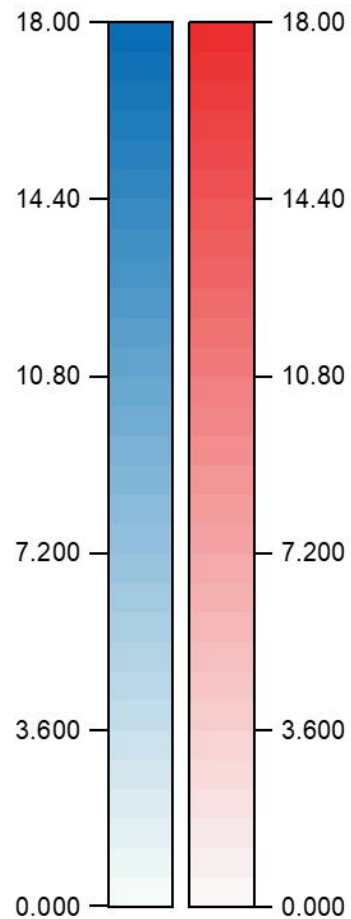
24-30



30-36



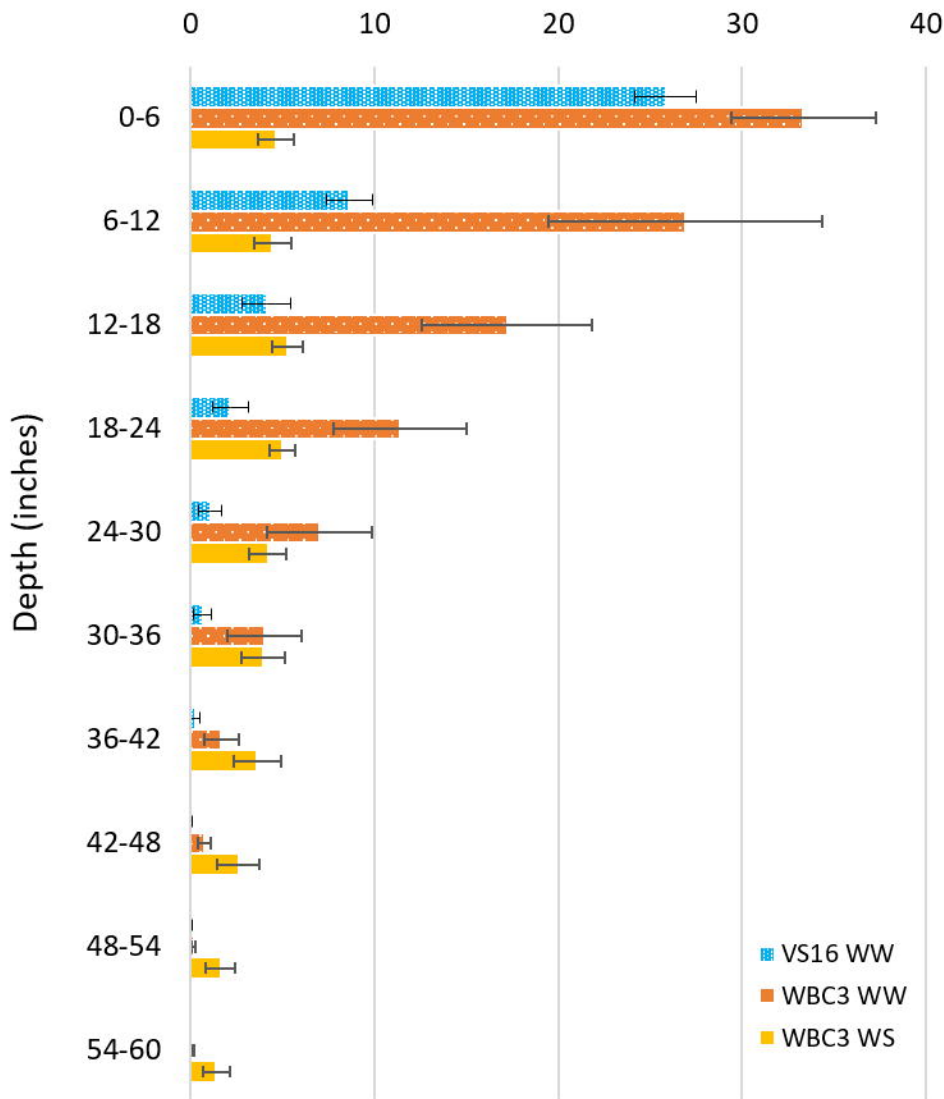
% of Total



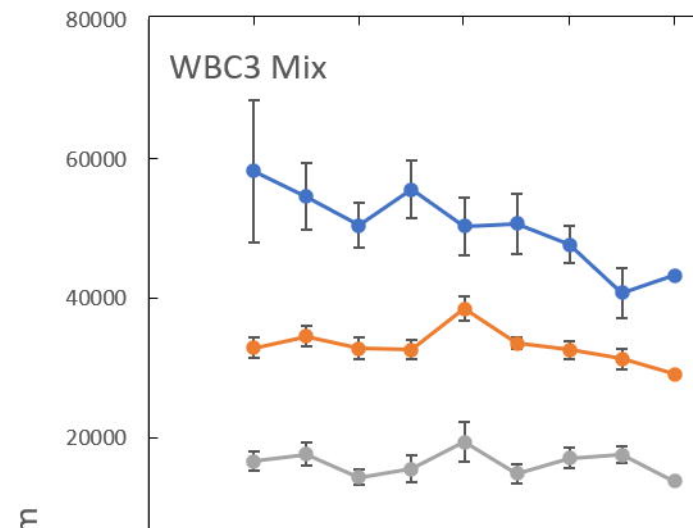
$R^2 = 0.88$

Biomass Allocation

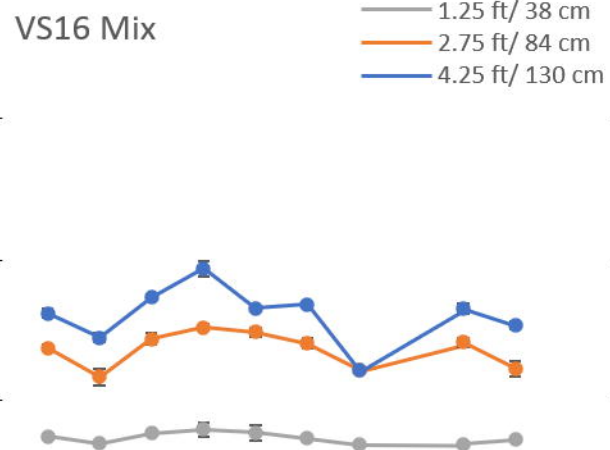
Weight (grams)



A

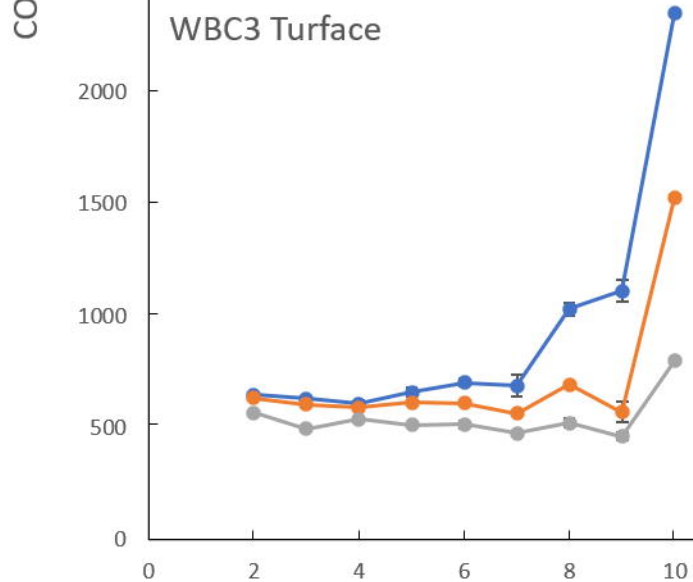


VS16 Mix

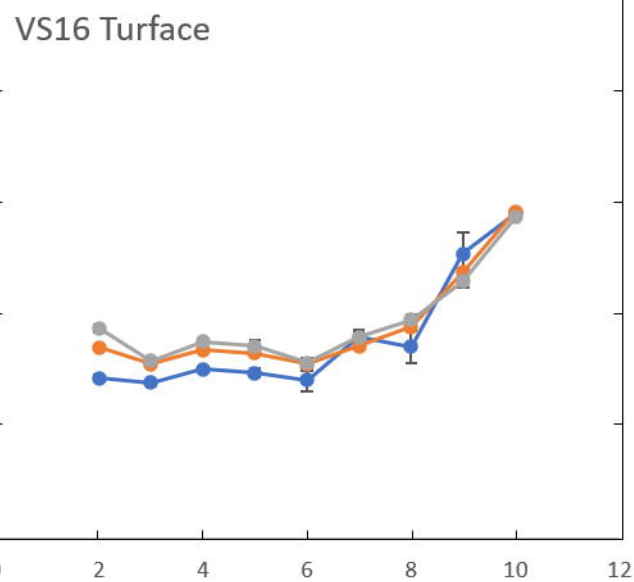


B

C

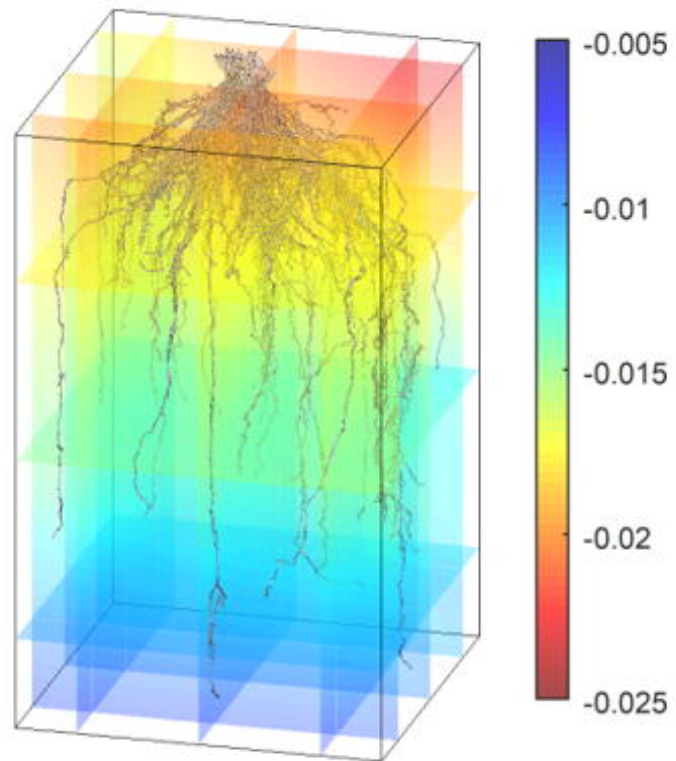
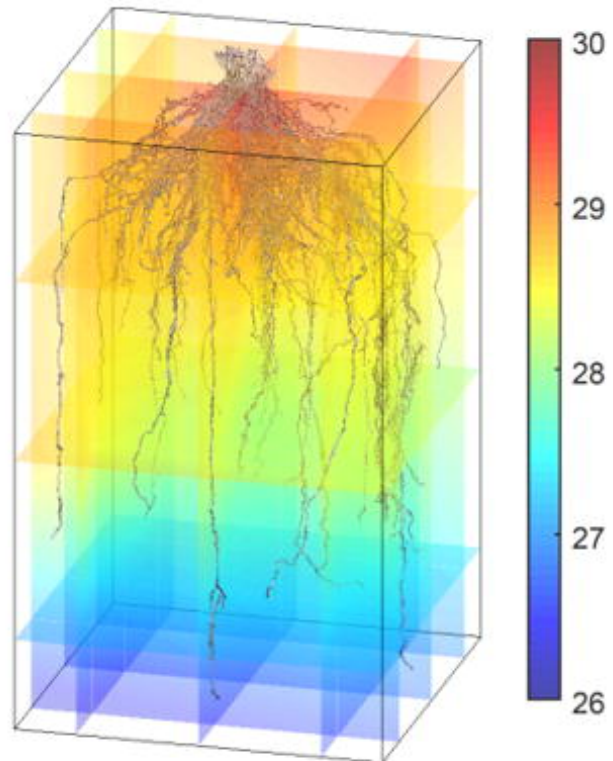
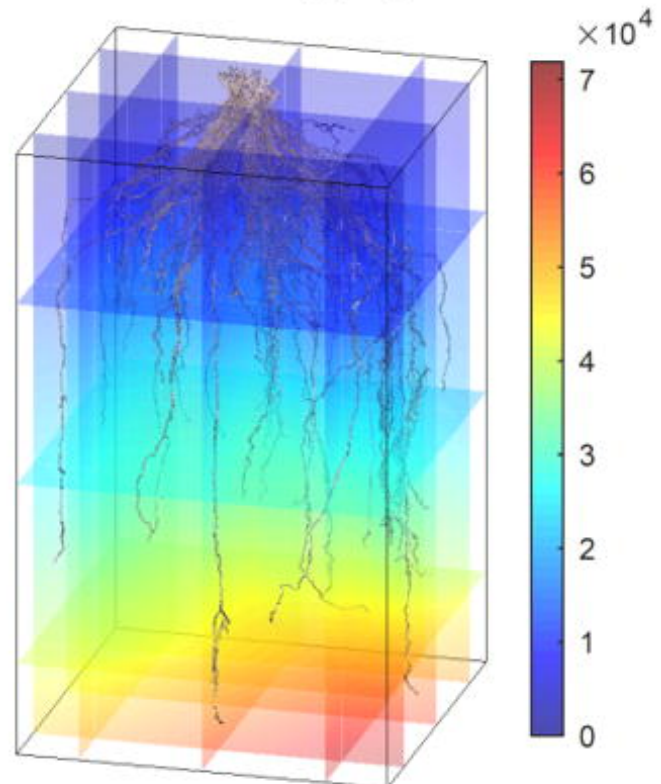


VS16 Turface

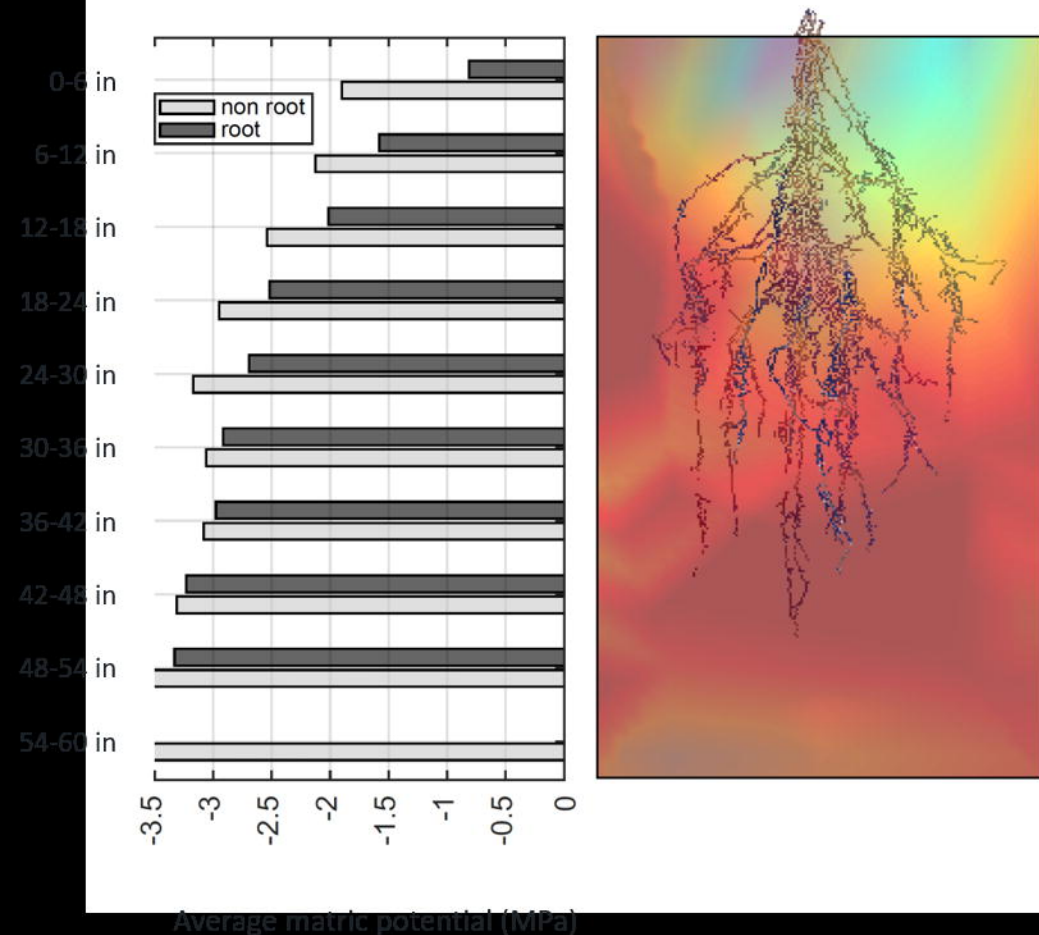


D

Weeks After Transplant

A Matric Potential (MPa)**B** Temperature ($^{\circ}\text{C}$)**C** CO_2 (ppm)

A. Water Stressed Matric Potential



B. Well Watered Matric Potential

