The Pennsylvania State University

Final Scientific/Technical Report

DEEPER: AN INTEGRATED PLATFORM FOR DEEPER ROOTS

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Project Team Members	Dr. Paul Heinemann, PSU, Dr. Alex Bucksch, U Ga, Dr. Shawn Kaeppler, U Wisconsin, Drs. Malcolm Bennett and Tony Pridmore, U Nottingham (UK)
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Public Executive Summary

Crops with deeper roots would have multiple benefits, including better drought tolerance, reduced requirement for nitrogen fertilizer, and better sequestration of atmospheric CO_2. **DEEPER** is an integrated platform of phenomic, genomic, and *in silico* technologies to generate maize lines with deeper roots. **DEEPER** is:

LEADER (*Leaf Elemental Accumulation from Deep Roots*) is a breakthrough technology to nondestructively measure rooting depth by using the plant itself as a sensor. LEADER uses handheld X-ray Fluorescence spectrometry to quantify foliar accumulation of elements that are differentially distributed in the soil profile. LEADER is nondestructive and is orders of magnitude cheaper, faster, and more precise than any competing assay of rooting depth in the field. LEADER is able to distinguish deep-rooted from shallow-rooted maize lines in the field without the need for costly and noisy soil coring.

RootRobot/DIRT3D, to automatically phenotype root architecture in any field, combining RootRobot, a mechatronics platform to excavate, clean, section, and image mature root crowns, with DIRT3D, software to quantify architectural traits in 3D.

Anatomics, a high-throughput platform to phenotype root anatomy, combining LAT 2.0, a technology for 3D imaging of root anatomy and composition, with RootScan3D, software to automatically extract 3D anatomical and cell wall composition metrics from LAT 2.0 output. Using this platform we discovered two novel root traits, *parenchyma cell wall thickness* and *multiseriate cortical sclerenchyma*, that improve rooting depth and drought tolerance in maize and wheat.

OpenSimRoot/Deep, software to simulate root interaction with hard subsoils. Using this platform we discovered novel concepts regarding how to increase crop rooting depth by modulating how individual root axes respond to hard soil.

DeepGenes, a toolkit of genes, parent lines, and genomic selection strategies to enable breeding hybrids with deeper roots. We discovered 3 novel root genes that increase rooting depth in maize and wheat.

DEEPER discovered novel root phenotypes for deeper rooting, and delivered validated ideotypes for deeper-rooted maize; novel technologies to rapidly assess root depth, root architecture and anatomy in field-grown plants; novel software tools for root modeling and 3D image analysis of root architecture and anatomy; and validated genes and genomic selection models to deploy traits for deeper rooting in maize breeding. Each **DEEPER** technology is transformative in its own right, and exceeds existing technologies. They are mutually synergistic, deployable for field-grown plants, and are ready for application. The phenotyping and modeling technologies are readily applicable to many crops, and genetic leads in maize may have utility in other grasses. Taken as a whole they represent a transformative platform to develop deeper-rooted crops, with greater drought tolerance, reduced fertilizer requirement, and greater carbon sequestration.

Acknowledgements

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Accomplishments and Objectives

This award allowed The DEEPER team to demonstrate a number of key objectives. The focus of the project was creating tools, resources, and devices to support the breeding of deeper-rooted maize and other crops.

A number of tasks and milestones were laid out in Attachment 3, the Technical Milestones and Deliverables, at the beginning of the project. The actual performance against the stated milestones is summarized here:

Table 1. Key Milestones and Deliverables.

Task 1: LEADER	
M1.1 Soil mapping	Q4: Results will be sufficient to relate soil trace element availability with soil depth to 60 cm at the spatial resolution of 10 square meters of land area. Actual Performance: Q6. Elemental profiles of soils at main experimental sites quantified.
M1.2 Leaf Samples	Q3: Submit an optimized sampling protocol that maximizes elemental signal by XRF while showing the greatest correlation with root depth. Develop methods with correlation greater than r=0.5 between leaf element content indices and root depth profiles. Actual Performance: Q8. Correlations of XRF leaf signals with root depth from coring were generated starting Q4 and improved with subsequent field sampling through Q20. Correlation of leaf XRF signals and rooting depth attained $r^2 = 0.76$, which is 300% greater than our goal of $r^2 = 0.25$.
M1.3 Optimize XRF protocol	Q4: XRF values are highly correlated with ICP values (r > 0.8) with greater than 2x faster throughput (including sample preparation) at < 20% of the marginal cost of ICP analysis for 4 or more elements. Actual Performance: Q8: Milestone completed. Correlation (r^2) of many elements of interest between XRF and ICP is greater than 0.9, in some cases this is lower due to detection limits of XRF technology employed. XRF is an acceptable alternative to ICP.
M1.4 Grow maize w/ tracers; Define LEADER protocols 2nd year	Q7: Definition of LEADER protocols will integrate local site characteristics of trace element availability with soil depth, optimized leaf sampling, and optimized XRF settings. Actual Performance: Q10: Milestone completed, continued refinement through Q20.
M1.5 Compile hybrid depth and elemental profile data	Q7: Develop report of 20 hybrids with root length density and trace element availability at 10 cm increments to 60 cm depth. Actual Performance: Q7: Milestone completed, continued refinement through Q20.
M1.6 Validate LEADER	Q11: Develop ability to predict utility of LEADER protocols given soil taxonomic identity at great group level. Actual Performance: Q11: Milestone completed, continued refinement through Q20 for major maize production soil taxa.
M1.7 Deploy LEADER	Q19: Correlations between XRF and LEADER results is r = 0.5 or better. Actual Performance: Q19: Milestone completed, r = 0.87

Task 2: RootRobot/DIRT 3D	
M2.1 RootRobot Robotic function	Q5: In lab simulation of digging, cleaning, and slicing of 20 stalks. Target of 100% locating and digging target and removing 90% soil. Actual Performance: Q10: Milestone completed, 20 stalks were successfully targeted, sliced, and cleaned by the unit.
M2.2 Optical Imaging system assembled	Q8: Industrial video cameras are added to the platform and capable of capturing still images. Actual Performance: Q10 Milestone completed. First a multicamera-prototype was designed by the UGA team of Bucksch. Then the PSU team of Heinemann designed, built and tested an industrial imaging chamber with the multi-camera imaging system. 10 high resolution cameras were set on a 90° arc, and the chamber rotated around the inverted stalk over 360° in 1° increments, resulting in 3600 images per stalk at this stage.
M2.3 Field Test RootRobot with Optical Imaging System	Q8: Determination is made for proper image angles or rotating plant. Imaged 20 crowns Actual Performance: Q10: Milestone completed. Cameras angles were adjusted to ensure complete coverage of the stalk as the chamber rotated around the stalk as described in M2.2. Hundreds of crowns were successfully imaged.
M2.4 3D model generated with OpenSimRoot	Q2: 30 genotypes of the LEADER Test Panel are simulated in SimRoot and all architectural traits are available as distributions per genotype (~30 simulations per genotype). Actual Performance: Q2: Field sampling was more extensive such that a 100-sample test set of real roots could be collected to have a preserved standard test set of real soil grown roots throughout the whole project.
M2.5 3D Model generated with X-Ray CT	Q4: Analyze 50 plants by X-Ray CT with 5 replications of 10 lines in the DEEP Genes panel. Actual Performance: Q4: Availability problems at the Houndsfield XRay facility during the pandemic inhibited this task as planned. Instead, we used the DIRT 2D system and single images from the manually taken 3D test data sets as projections in this iterative improvement process.
M2.6 DIRT3D year 1 performance	Q8: Our aim is to achieve r>=0.7 for these manually measured traits. The algorithm is also deployed (without terahertz support) to the DIRT imaging platform. Actual performance: Q: Milestone achieved. The correlation for all manually measured traits was r>=0.9. This result was substantially better than we ever imagined for an optical system.

M2.8 THz camera mounted	Q10: Camera successfully mounted on RootRobot. Actual Performance: Q15: Milestone achieved. The project was modified to produce a factor 100 cheaper system after early successes with a purely optical system without THz support. Therefore, updated optical cameras were installed.
M2.9 Develop terahertz algorithms	Q12: Our goal is to achieve a r=0.7 correlation for root counts, eccentricity, and root width on 100 roots imaged with the RootRobot system. Actual performance: Q12: Milestone achieved after project modification. The cheaper optical system had much better performance than anticipated. System cost reduced by factor 100 compared to the planned terahertz system. Correlation for all manually measurable traits including highly occluded traits inside the root crown was r>=0.9. To achieve these correlations a new calibration method for multi-camera systems was developed.
M2.11 Conduct RootRobot field trials in diverse soils	Q11: Unit should successfully dig, clean, and slice 100% of samples in both soil types at dry and medium conditions, and 90% of samples in wet conditions. Actual Performance: Q16: Milestone not achieved. Field tests were performed but the unit was unable to successfully dig, clean, and slice the samples under field conditions due to hardware issues (sensors, robustness of locating mechanism).
M2.12 Discovery additional Root architectural traits	Q19: Discover at least one novel trait with RootRobot and DIRT3D Actual Performance: Q12, milestone achieved. Publication of discovery of zmCIPK15 controlling root growth angle of nodal roots 3 and 4, associated with deeper rooting and better N capture of maize grown in low N soil. This discovery utilized ongoing evolution of DIRT and shovelomics pipelines.
Task 3: Anatomics	
M3.1 Create LAT 2.0 image database	Q2: a semi-automatic tool will be developed to support annotation of LAT 2.0 images. The database will consist of 1000 to 10000 images. Actual Performance: Milestone achieved. Cellset, a pre-existing University of Nottingham software tool was revised to ingest and semi automatically annotate LAT 2.0 images. A database of 100 images was curated, 1000-10,000 was not required to achieve the goals stated in Milestone 3.3 and 3.5. By selecting unique and varied images we were able to reduce the annotation number required.
M3.2 Assembly of LAT 2.0	Q4: Automation in LabView will increase throughput by 2-3x over existing LAT. LAT in video mode can capture 1800 high quality frames per minute each with sub-micron x, y, and z

	resolution for reconstruction into a 3D image stack. LAT 2.0 will have at least 50% greater throughput and will generate sharper images, especially of very thin and very thick tissue sections, compared with existing LAT. Actual Performance: Q4, Milestone achieved.
M3.3 CNN tool milestone	Q4: Develop CNN based segmentation of root anatomy capable of detecting have 80% of input pixels classified in line with direct human analysis. Actual Performance: A CNN based on the Hourglass architecture was developed and trained on LAT images. The CNN detects 81.6% of pixels correctly when compared with human analysis. Milestone achieved.
M3.4 Add Hyperspectral imaging to LAT 2.0	Q8: Our goal is to identify specific tissues and cell types enriched in specific cell wall constituents at > 2x greater throughput than conventional staining methods. Actual Performance: Q16, milestone achieved. Automated multispectral analysis capable of detecting composition of root tissue such as lignin in MCS with many orders of magnitude faster throughout and reduced cost compared with staining methods.
M3.5 Dvlp RootScan3D methods and tools	Q8: Tools will mix heuristic and learned methods for identifying key relationships between objects. These tools will increase analytical throughput by >2x compared with existing tools while capturing > 80% of the features identifiable by direct visual analysis. Actual Performance: The new rootscan tool has a throughput of 6x previous automated methods. The tool retains it's 81.6% accuracy at this throughput. Milestone achieved.
M3.6 Discover/validate novel traits	Q19: Anatomical trait report demonstrating achieved goal of 20% increase in D95. Actual Performance: Q15, milestone achieved. We discovered a novel anatomical trait called *multiseriate cortical sclerenchyma* in cereal roots. Genotypes of maize, wheat, barley, and other cereals that possess MCS have greater lignin content and hence stronger, stiffer roots that are better able to penetrate hard soil and are therefore able to achieve greater root depth and better plant growth in compacted soils in the field. From 30 to 50% of modern cereal lines have this trait but it is lacking in ancient crop landraces and wild ancestors, suggesting that it is an adaption to mechanized agriculture. We identified a candidate gene associated with variation for this trait.
Task 4: OpenSimRoot/deep	

M4.1 Develop soil physics module for OpenSimRoot (OSR)	Q4: Soil hardness in simulations correlated to actual soil hardness at > r = 0.5 with a spatial precision of 1 cm^3. Actual Performance: Q8 milestone achieved.
M4.2 Develop hard soil penetration simulation algorithms	Q8: SimRoot achieves simulation of the dynamics of root penetration of soil as a function of soil hardness with sufficient utility to guide empirical research. This task has been completed successfully if > 50% of the insights generated are subsequently validated empirically. Actual Performance: Q8 milestone achieved. Insights validated (see M.5 below).
M4.3 Deploy SimRoot/deep; Optimize w/ BORG; Integration	Q12: Reported fitness landscape from BORG for the genetic optimization of 20 root phenes interacting with soil hardness. Actual Performance: Milestone partially achieved. BORG optimization and statistical machine learning successfully employed for root depth and tolerance to low N and P but not including new soil hardness feature of OSR.
M4.4 Novel traits and phenotypes	Q19: Novel phenes, phene states, and ideotypes will be capable of increasing root depth, water capture, or N capture by 5% relative to standard commercial phenotypes. Actual Performance: Q19, milestone achieved. Maize root phenotypes identified by OSR having contrasting reaction to soil hardness resulting in 55% increase in rooting depth and 20% increase in N capture.
M4.5 Field validation of phenotypes	Q19: Metric of success: phenes, phene states, and their interactions suggested as useful in improving root depth by SimRoot will be validated in field studies with >50% success. Actual Performance: Q19, milestone achieved. OSR correctly predicted that stiffening of axial roots should increase rooting depth in maize- this was validated by results with MCS.
Task 5: Deepgenes	
M5.1 Year 1: evaluate major gene isoline pairs	Q4: One or more major gene isoline pairs increase root depth by 10% relative to the control. Actual Performance: Q4: milestone partially achieved: isolines for zmCIPK15 and zmbHLH121 evaluated. Increase in root depth sometimes noted. Given field and seasonal variation, more validation needed.
M5.2 Year 2: evaluate major gene isoline pairs	Q8: One or more major gene isoline pairs increase root depth by 10% relative to the control. Actual Performance: Actual Performance: Q8: milestone partially achieved: isolines for zmCIPK15 and zmbHLH121 evaluated. Increase in root depth sometimes noted. Given field and seasonal variation, more validation needed.

M5.3 Year 3: evaluate major gene isoline pairs	Q12: One or more major gene isoline pairs increase root depth by 10% relative to the control. Actual Performance: Actual Performance: Q12: milestone partially achieved: isolines for zmCIPK15 and zmbHLH121 evaluated. Increase in root depth sometimes noted. Given field and seasonal variation, more validation needed.
M5.4 Year 4: evaluate major gene isoline pairs	Q19: One or more major gene isoline pairs increase root depth by 10% relative to the control. Actual Performance: Actual Performance: Q19: milestone achieved: isolines for zmCIPK15 and zmbHLH121 increase in root depth (D_{75}) in some years and some environments. GxE not fully understood. While zmCIPK15 isolines did not change whole plant rooting depth as shown by coring at a single time point, they did have steeper root growth angles at specific nodal positions, and did take up more deep N^{15} and had greater biomass in limited N plots.
M5.2.2 Seed produced for year 2	Q4:100% of required seed is produced to support subsequent year trials. Actual Performance: Q4 milestone achieved. 100% of required seed produced.
M5.2.3 Seed produced for year 3	Q8: 100% of required seed is produced to support subsequent year trials. Actual Performance: Q8 milestone achieved. 100% of required seed produced.
M5.2.4 Seed produced for year 4	Q12: 100% of required seed is produced to support subsequent year trials. Actual Performance: Q12 milestone achieved. 100% of required seed produced.
M5.6 Eval Hybrid Panel & GWAS; 300 hybrid/yr	Q8: Accomplishment documented yearly by completion of planting, root trait evaluation, and harvest. Actual Performance: Q8 milestone partially achieved. Planting and harvest were completed with success. Root trait evaluation depended on LEADER results, and we did it in a subset of the trials. Test evaluations in optimal, limited water, limited nitrogen, and limited water and nitrogen were realized to infer root traits and as a potential indicator for root depth. Also, we collected agronomic traits, grain yield, and essential traits in a maize breeding program.
M5.7 Eval Hybrid Panel & GWAS; 300 hybrid/yr	Q12: Accomplishment documented yearly by completion of planting, root trait evaluation, and harvest. Actual Performance: Q12 milestone partially achieved. Planting and harvest were completed with success. Root trait evaluation depended on LEADER results, and we did it in a subset of the trials. Test evaluations in optimal, limited water, limited nitrogen, and limited water and nitrogen were realized to infer root traits and as a potential indicator for root depth.

	Also, we collected agronomic traits, grain yield, and essential traits in a maize breeding program.
M5.8 Evaluate 400 bi-parental hybrids for Genomic Selection	Q8: Accomplishment documented yearly by completion of planting, root trait evaluation, and harvest. Actual Performance: Q8 milestone partially achieved. Planting and harvest were completed with success. Root trait evaluation in a subset of the trials. One challenge of the project was developing the phenotyping platform at the same time we were evaluating the hybrids to be used in the genomic selection. Similar to the Diverse hybrids, we collected agronomic traits, grain yield, and essential traits in a maize breeding program. We did test evaluations in optimal and limited nitrogen to infer root traits and as a potential indicator for root depth.
M5.9 Evaluate 400 bi-parental hybrids for Genomic Selection	Q12: Accomplishment documented yearly by completion of planting, root trait evaluation, and harvest. Actual Performance: Q12 milestone partially achieved. Planting and harvest were completed with success. Root trait evaluation in a subset of the trials. One challenge of the project was developing the phenotyping platform at the same time we were evaluating the hybrids to be used in the genomic selection. Similar to the Diverse hybrids, we collected agronomic traits, grain yield, and essential traits in a maize breeding program. We did test evaluations in optimal and limited nitrogen to infer root traits and as a potential indicator for root depth.
M5.10 Run predict model on 4,000 lines; ID top 100	Q12: The model that incorporates phene related information has a prediction accuracy that is 20% higher than models that do not include phene specific information. Actual Performance: Based on the preliminary results of LEADER trials and previous studies, the hypothesis is that most tolerant hybrids, i.e., higher yielding under limited conditions (stress), have deeper roots. Without root phene-related information, we used the Anthesis-Silking interval (ASI) under limited nitrogen (N) and water conditions as an indirect predictor of grain yield (GY). ASI has higher heritability than grain yield under low water and low N, and it correlated to N and water stress tolerance. However, it did not increase the prediction accuracy in our experiment. The prediction accuracy achieved using ASI as a predictor was comparable to GY only in two scenarios, when the predictors were estimated under low water, and the response was GY under low water and when the predictors were estimated under low water and low N, and the response was GY under low water and low N in 2019. Although there was an increase of 20% and 6.7% in prediction accuracy, respectively, there was overlap among the standard error of the estimates.

M5.11 Validate GS approach by evaluating top 100 hybrids	Q19: Hybrids are identified that increase root depth 30% relative to control hybrids which are the original crosses from which our doubled haploid populations are derived. Actual Performance: In the absence of root depth measurements, we used LEADER findings to guide what traits would be informative about root depth. Here we also followed the hypothesis that most tolerant hybrids, i.e., higher yielding under limited conditions, have deeper roots. We identified hybrids that performed well under nonlimited and limited conditions (above the experiment mean in both conditions) and had a short anthesis-silking interval. The development of the phenotyping platform at the same time as the evaluation of the hybrids to be used in the GS was challenging because we needed to receive root trait information to include in the models, and it was not possible to collect root depth and root related traits in a large number of plots. Besides all the agronomic and yield traits collected in nonlimited, low water, low nitrogen, and low water and low nitrogen conditions and used as a potential indicator for root depth, we collected leaf samples for XRF analysis (X-Ray fluorescence technology to identify deep-rooted genotypes by using soil elemental gradients to determine root depth). The leaves samples are being processed but still need to be finalized.

Modeling

In this project we substantially expanded the capabilities of *OpenSimRoot (OSR)*, currently the most powerful and feature-rich model of root/soil interactions at the root/whole plant/stand scale. In this project we added the capability of OSR to simulate how roots interact with soil mechanical impedance in a spatiotemporally precise and dynamic manner, including soil hardening as a result of soil water status resulting from plant water uptake and varying precipitation regimes. This work has been published in 2 refereed journal articles:

1) Strock CF, H Rangarajan, CK Black, ED Schäfer, JP. Lynch. 2022. Theoretical evidence that root penetration ability interacts with soil compaction regimes to affect nitrate capture. Annals of Botany 129, 315–330 https://doi.org/10.1093/aob/mcab144.
2) Schäfer E, I Ajmera, E Farcot, MR Owen, LR Band, JP Lynch. 2022. *In silico* evidence for the utility of parsimonious root phenotypes for improved vegetative growth and carbon sequestration under drought. BioRxiv 10.1101/2022.08.04.502508, Frontiers in Plant Science DOI: 10.3389/fpls.2022.1010165

Project Activities

Crops with deeper roots would have multiple benefits, including better drought tolerance, reduced requirement for nitrogen fertilizer, and better sequestration of atmospheric CO_2. A major obstacle to the development of deeper rooted crops is that rooting depth is difficult to measure in field conditions. The goal of the DEEPER project was to develop a set of synergistic tools to support the breeding of deeper-rooted crops, focusing on maize as an example. These tools included **LEADER**, a revolutionary method to rapidly and nondestructively measure root depth in the field; **RootRobot/DIRT3D** to automatically measure root architecture in the field in 3D; **Anatomics** to rapidly measure root anatomy and composition, ***OpenSimRoot/Deep***, software to simulate root interaction with hard soils; and **DeepGenes** a set of genetic tools to enable breeding maize with deeper roots. **DEEPER** discovered novel root phenotypes for deeper rooting, and delivered validated ideotypes for deeper-rooted maize; novel technologies to rapidly assess root depth, root architecture and anatomy in field-grown plants; novel software tools for root modeling and 3D image analysis of root architecture and anatomy; and validated genes and genomic selection models to deploy traits for deeper rooting in maize breeding. Each **DEEPER** technology is transformative in its own right, and exceeds existing technologies. They are mutually synergistic, deployable for field-grown plants, and are ready for application. The phenotyping and modeling technologies are readily applicable to many crops, and genetic leads in maize may have utility in other grasses. Taken as a whole they represent a transformative platform to develop deeper-rooted crops, with greater drought tolerance, reduced fertilizer requirement, and greater carbon sequestration.

Project Outputs (input from Alex, Malcolm, SMK, TP)

A. *Journal Articles*

Lima, D.C., N. de Leon, and S.M. Kaeppler. 2022. Utility of anthesis-silking interval information to predict grain yield under water and nitrogen limited conditions. *Accepted Crop Science.*

Marshall-Colon A, SP Long, DK Allen, G Allen, DA Beard, B Benes, AJ Christensen, DJ Cox, JC Hart, PM Hirst, K Kannan, DS Katz, JP Lynch, AJ Millar, B Panneerselvam, ND Price, D Raila, RG Shekar, S Shrivastava, D Shukla, V Srinivasan, M Stitt, EO Voit, Y Wang, X Yin, X-G Zhu. 2017. Crops in silico: Generating virtual crops using an integrative and multi-scale modeling platform. Frontiers in Plant Science https://doi.org/10.3389/fpls.2017.00786.

Postma, JA, C Kuppe, MR Owen, N Mellor, M Griffiths, MJ Bennett, JP Lynch, M Watt. 2017. *OpenSimRoot*: Widening the scope and application of root architectural models. New Phytologist, doi: 10.1111/nph.14641

Marshall-Colon A, SP Long, DK Allen, G Allen, DA Beard, B Benes, AJ Christensen, DJ Cox, JC Hart, PM Hirst, K Kannan, DS Katz, JP Lynch, AJ Millar, B Panneerselvam, ND Price, D Raila, RG Shekar, S Shrivastava, D Shukla, V Srinivasan, M Stitt, EO Voit, Y Wang, X Yin, X-G Zhu. 2017. Crops in silico: Generating virtual crops using an integrative and multi-scale modeling platform. Frontiers in Plant Science https://doi.org/10.3389/fpls.2017.00786.

Alexander Bucksch et al (37 authors, including JP Lynch). 2017. Morphological plant modeling: Unleashing geometric and topological potential within the plant sciences. Frontiers in Plant Science https://doi.org/10.3389/fpls.2017.00900

Lynch JP 2018. Rightsizing root phenotypes for drought resistance. J Experimental Botany, Vol. 69, No. 13 pp. 3279–3292, 2018

Lynch JP. 2019. Root phenotypes for improved nutrient capture: an underexploited opportunity for global agriculture. Invited Tansley Review, New Phytologist 223: 548–564 doi: 10.1111/nph.15738.

Hall B, A Lanba, JP Lynch. 2019. Three-dimensional analysis of biological systems via a novel laser ablation technique. J. Laser Appl. 31, 022602.

Strock C, HM Schneider, T Galindo-Castañeda, BT Hall, B Van Gansbeke, DE Mather, MG Roth, MI Chilvers, X Guo, KM Brown, JP Lynch. 2019. Laser ablation tomography for visualization of root colonization by edaphic organisms. Journal of Experimental Botany 70: 5327–5342.

Yang JT, HM Schneider, KM Brown, JP Lynch. 2019. Genotypic variation and nitrogen stress effects on root anatomy in maize are node-specific. Journal of Experimental Botany 70 5311–5325.

Galindo-Castañeda T, KM Brown, G Kuldau, G Roth, N Wenner, S Ray, JP Lynch. 2019. Root cortical anatomy is associated with differential pathogenic and symbiotic fungal colonization in maize. Plant, Cell, and Environment 42:2999-3014.

Le Marié C, K-H Camp, A Strigens, JP Lynch, LM York, M Malosetti, A Hund. Shovelomics root traits assessed on the EURoot maize panel are highly heritable across environments but show low genotype-by-nitrogen interaction. Euphytica 215: 173

Benes B, K Guan, M Lang, SP Long, JP Lynch, A Marshall-Colón, B Peng, J Schnable, L Sweetlove, M Turk 2020 Multiscale computational models can guide experimentation and targeted measurements for crop improvement. The Plant Journal 103:21-31

Schneider HM, Klein SP, Hanlon MT, Brown KM, Kaeppler SM, Lynch JP. Genetic control of root anatomical plasticity in maize. 2020. The Plant Genome 13 https://doi.org/10.1002/tpg2.20003

Schneider HM, SP Klein, MT Hanlon, EA Nord, SM Kaeppler, KM Brown, A Warry, R Bhosale, JP Lynch. 2020. Genetic control of root architectural plasticity in maize. J Experimental Botany 71:3185-3197 https://doi.org/10.1093/jxb/eraa084

Vanhees DJ, KW Loades, GA Bengough, SJ Mooney, JP Lynch. 2020. Root anatomical traits contribute to deeper rooting of maize (*Zea mays* L.) under compacted field conditions. J Experimental Botany 71 4243–4257 doi:10.1093/jxb/eraa165

Klein SP, HM Schneider, AC Perkins, KM Brown, JP Lynch. 2020. Multiple integrated root phenotypes are associated with improved drought tolerance. Plant Physiology 83, 1011–1025, www.plantphysiol.org/cgi/doi/10.1104/pp.20.00211

Schneider HM, JP Lynch. 2020. Should root plasticity be a crop breeding target? Frontiers in Plant Science 11: 546. doi: 10.3389/fpls.2020.00546

Strock CF, JP Lynch. 2020. Root secondary growth: An unexplored component of soil resource acquisition. Annals of Botany 126:205–218, doi: 10.1093/aob/mcaa068

Schneider HM, JA Postma, J Kochs, D Pflugfelder, JP Lynch, D van Dusschoten. 2020. Spatio-temporal variation in water uptake in seminal and nodal root systems of barley plants grown in soil. Frontiers in Plant Science https://doi.org/10.3389/fpls.2020.01247

Strock, CF, JD Burridge, MD Niemiec, KM Brown, JP Lynch. 2020. Root metaxylem phenotypes interact with root architecture to regulate water use under drought stress. Plant, Cell Environment 1-19 https://doi.org/10.1111/pce.13875

Burridge JD, CK Black, EA Nord, JA Postma, JS Sidhu, LM York, JP Lynch. 2020. An analysis of soil coring strategies to estimate root depth. Plant Phenomics 3252703 https://doi.org/10.34133/2020/3252703

Schneider HM, CF Strock, MT Hanlon, DJ Vanhees, AC Perkins, IB Ajmera, JS Sidhu, SJ Mooney KM Brown, JP Lynch. 2021. Multiseriate cortical sclerenchyma enhance root penetration in compacted soils. PNAS 118 (6) e2012087118; https://doi.org/10.1073/pnas.2012087118.

Rangarajan H, JP Lynch. 2021. A comparative analysis of quantitative metrics of root architectural phenotypes. Plant Phenomics, Article ID 6953197 https://doi.org/10.34133/2021/6953197

Holland J et al. (40 authors, including JP Lynch). 2021. The importance of dominance and genotype-by-environment interactions on grain yield variation in a large-scale public cooperative maize experiment. G3 DOI: 10.1093/g3journal/jkaa050

Levin KA, MR Tucker, CF Strock, JP Lynch, DE Mather. 2021. Three-dimensional imaging reveals that positions of cyst nematode feeding sites relative to xylem vessels differ between susceptible and resistant wheat. Plant Cell Reports, 40:393-403 https://doi.org/10.1007/s00299-020-02641-w

Vanhees D, KW Loades, AG Bengough SJ Mooney, JP Lynch 2021. The ability of maize roots to grow through compacted soil is not dependent on the amount of roots formed. Field Crops Research 264: 108013

Pandey BK, G Huang, R Bhosale, S Hartman, L Jose, CJ Sturrock, OC Martin, M Karady, LACJ Voesenek, K Ljung, JP Lynch, KM Brown, WR Whalley, SJ Mooney, D Zhang, MJ Bennett. 2021. Plant roots sense soil compaction through restricted ethylene diffusion. Science 371:276-280. DOI: 10.1126/science.abf3013

Schneider HM, JT Yang, KM Brown, JP Lynch. 2021. Nodal root diameter and node number interact to influence nitrogen stress tolerance in maize (*Zea mays* L.). Plant Direct http://doi.org/10.1002/pld3.310

Lynch JP, CF. Strock, HM Schneider, JS Sidhu, I Ajmera, T Galindo-Castañeda, SP Klein, MT Hanlon. 2021. Root anatomy and soil resource capture. Plant and Soil (466),21-63 10.1007/s11104-021-05010-y

Lynch JP. 2021. Root biology in the 21st century: challenges and opportunities. Viewpoint. Annals of Botany, 128:i-ii. DOI: 10.1093/aob/mcab062

Perkins A, JP Lynch Increased seminal root number associated with domestication improves nitrogen and phosphorus acquisition in maize seedlings. Annals of Botany 128(4): 453–468.

Liu, S, C Barrow, M Hanlon, JP Lynch, A Bucksch. 2021. DIRT/3D: 3D phenotyping for field-grown maize (*Zea mays*). Plant Physiology 187:739-757.

Saengwilai P, J Chimungu, H Rangarajan, C Strock, J Salungyu, JP Lynch. 2021. Root hair phenotypes influence nitrogen acquisition in maize. Annals of Botany,128:849-858 https://doi.org/10.1093/aob/mcab104

Lynch JP Harnessing root architecture to address global challenges. 2021. Plant Journal doi: 10.1111/tpj.15560

Rangarajan H, D Hadka, P Reed, JP Lynch. 2022. Multi-objective optimization of root phenotypes for nutrient acquisition using evolutionary algorithms. The Plant Journal April 15 2022 https://doi.org/10.1111/tpj.15774

Vanhees D, HM Schneider, JS Sidhu, KW Loades, AG Bengough MJ Bennett, KM Brown, SJ Mooney, JP Lynch. 2022. Genotypic variation in soil penetration by maize roots is negatively related to ethylene-induced thickening. Plant Cell Environment 45:789-804 DOI: 10.1111/pce.14175

Schneider HM, Vai Sa Nee Lor, MT Hanlon, A Perkins, SM Kaeppler, R Bhosale, AN Borkar, X Zhang, J Rodriguez, A Bucksch, MJ Bennett, KM Brown, JP Lynch. 2022. Root angle in maize influences nitrogen capture and is regulated by *ZmCIPK15.* Plant, Cell, & Environment 45: 837-853 https://doi.org/10.1111/pce.14135

Ajmera I, Henry A, Radanielson AM, Klein SP, Ianevski A, Bennett MJ, Band LR, JP Lynch. 2022. Integrated root phenotypes for low nitrogen tolerance in rice. Plant, Cell & Environment 45: 805-822. http://dx.doi.org/10.1111/pce.14284

Lynch JP, SJ Mooney, CF Strock, HM Schneider. 2022. Future roots for future soils. Plant, Cell & Environment 45:620-636, https://doi.org/10.1111/pce.14213

Guang-Jun Wu, Xucun Jia, Christopher Strock, Shu-Ting Dong, Ji-Wang Zhang, Bin Zhao, Jonathan P. Lynch, Peng Liu. 2022. Root anatomical phenotypes related to growth under low nitrogen availability in maize (*Zea mays* L.) hybrids. Plant Soil 10.1007/s11104-022-05331-6

Strock CF, HM Schneider, JP Lynch. 2022. *Anatomics*: high-throughput phenotyping of plant anatomy. TIPS https://doi.org/10.1016/j.tplants.2022.02.009

Galindo-Castañeda T, JP Lynch, J Six, M Hartmann. 2022. Improving soil resource uptake by plants through capitalizing synergies between root architecture and anatomy and root-associated microorganisms. Frontiers in Plant Science, in press accepted Feb 15

Strock CF, H Rangarajan, CK Black, ED Schäfer, JP. Lynch. 2022. Theoretical evidence that root penetration ability interacts with soil compaction regimes to affect nitrate capture. Annals of Botany 129, 315–330 https://doi.org/10.1093/aob/mcab144

Lopez Valdivia Ivan Alden Perkins, Hannah Schneider, Miguel Vallebueno-Estrada, James Burridge, Eduardo González-Orozco, Aurora Montufar, Rafael Montiel, Jonathan Lynch, Jean-Philippe Vielle-Calzada 2022. Gradual domestication of root traits in the earliest maize from Tehuacan. PNAS Vol. 119 No. 17 e2110245119

Martínez-Gómez J, TAM Atluri, IJ Rose, AJ Holliday, CF Strock, JP Lynch, WB Miller, DW Stevenson, CD Specht. 2022. Developmental morphology and anatomy shed light on both parallel and convergent evolution of the umbellate inflorescence in Monocots, underlied by a new variant of metatopy. Frontiers in Plant Science, 29 April 2022 DOI: 10.3389/fpls.2022.873505

Clement C, HM Schneider, DB Dresbøll, JP Lynch, K Thorup-Kristensen. 2022. Root and xylem anatomy varies with root length, root order, soil depth, and environment in intermediate wheatgrass *Thinopyrum intermedium* and Alfalfa *Medicago sativa:* Implications for water uptake. AOB accepted April 3 2022

Lynch JP. The importance of edaphic stress interactions for plant function: important yet poorly understood drivers of plant production in future climates. Field Crops Research Volume 283, 1 July 2022, 108547 https://doi.org/10.1016/j.fcr.2022.108547

Fusi et al (33 coauthors, including JP Lynch) 2022. Root angle is controlled by *EGT1* in cereal crops employing a novel anti-gravitropic mechanism PNAS 119 (31) e2201350119

Sidhu, JS, I Ajmera, S Arya, JP Lynch. RootSlice: functional structural modeling of root anatomy. BioRxiv June 29 2022.10.1101/2022.06.29.498145

Schafer, Ernst, Markus R. Owen, Leah R. Band, Etienne Farcot, Malcolm J. Bennett, Jonathan P. Lynch. 2022. Modelling root loss reveals impacts on nutrient uptake and crop development in press Plant Physiology July 26 2022

Schäfer E, I Ajmera, E Farcot, MR Owen, LR Band, JP Lynch. 2022. *In silico* evidence for the utility of parsimonious root phenotypes for improved vegetative growth and carbon sequestration under drought. BioRxiv 10.1101/2022.08.04.502508, Frontiers in Plant Science DOI: 10.3389/fpls.2022.1010165

Liu, Suxing Bonelli, et al. "Comparison of open-source image-based reconstruction pipelines for 3D root phenotyping of field-grown maize." *2022 NAPPN Conference Proceedings*. 2022 *(an extended journal version of this proceedings paper will be published in The Plant Phenome Journal Special Issue of the NAPPN 2022 Conference)*

Knapp-Wilson, Jordan, et al. "Imaging and Quantitative Analysis of Peach Tree Branching Index Via Treeqsm." Acta Horticulturae. Vol. 56. No. 9, American Society for Horticultural Science, 2021.

C. Status Reports

Pietrzyk, Peter, Liu, Suxing. "RootRobot: Camera Calibration Guide." *Internal UGA-PSU document*. 2021

D. Media Reports

Known TV and Live Stream appearances:

CyVerse Webinar: https://www.youtube.com/watch?v=4s3L1vDANWs

Farm Monitor: https://www.youtube.com/watch?v=olJFwIi7CK0

Known News Outlets:

Technology Networks: https://www.technologynetworks.com/applied-sciences/news/adapting-roots-to-a-hotter-climate-could-reduce-pressure-on-food-supply-351471

Phys.org: https://phys.org/news/2021-07-roots-hotter-planet-ease-pressure.html

Mirage News: https://www.miragenews.com/adapting-roots-to-hotter-planet-could-ease-604822/

Western Producer: https://www.producer.com/news/new-3d-technology-helps-unlock-plants-root-trait-secrets/

Science Daily: https://www.sciencedaily.com/releases/2021/07/210729143426.htm

[If not provided previously, attach or send copies of any public releases to the DOE Program Manager identified in Block 15 of the Assistance Agreement Cover Page]

E. Invention Disclosures

XXXXXXXXX

F. Patent Applications/Issued Patents

XXXXXXXXX

G. Licensed Technologies

XXXXXXXXX

H. Networks/Collaborations Fostered

North American Plant Phenotyping Network, International Plant Phenotyping Network

I organized the yearly conference in Athens during the Project.

Anatomics In Pearl Millet: Improving Yield And Drought Tolerance Of A Major Dietary Staple Food In The Sahel

Royal Society International collaborative award with IRD (France) and ISRA (Senegal)

I. Websites Featuring Project Work Results

- www.plantit.cyverse.org
- www.computational-plant-science.org
- https://www.tacc.utexas.edu/-/adapting-roots-to-a-hotter-planet-could-ease-pressure-on-food-supply
- https://beta.nsf.gov/news/adapting-plant-roots-hotter-planet-could-help-ease
- https://research.uga.edu/news/adapting-roots-to-a-hotter-planet-could-ease-pressure-on-food-supply/
- https://idfs.uga.edu/projects/

J. Other Products (e.g. Databases, Physical Collections, Audio/Video, Software, Models, Educational Aids or Curricula, Equipment or Instruments)

- Prototype of the 3D root scanning system
- Calibration procedure for multi-camera systems
- All video outputs are collected here:
  http://computational-plant-science.org/joomla30/index.php/video-gallery
- Source codes and Documentation:
  DIRT/3D: https://github.com/Computational-Plant-Science/3D_model_reconstruction_demo
  PlantIT: https://github.com/Computational-Plant-Science/plantit

All video outputs are collected here: http://computational-plant-science.org/joomla30/index.php/video-gallery

Source codes and Documentation:

DIRT/3D: https://github.com/Computational-Plant-Science/3D_model_reconstruction_demo

PlantIT: https://github.com/Computational-Plant-Science/plantit

K. Awards, Prizes, and Recognition

NSF CAREER AWARD

Early Career Award of the North American Plant Phenotyping Network

Fred C. Davison Award

2022 MJB awarded ISSR Dundee Medal

2020 MJB elected to Royal Society

Follow-On Funding

Additional funding committed or received from other sources (e.g. private investors, government agencies, nonprofits) after effective date of ARPA-E Award.

Table 2. Follow-On Funding Received. **Source**	**Funds Committed or Received**
NSF CAREER AWARD	$1,137,000
Syngenta Research Contract	$ 100,000 p.a.
USDA NIFA	$ 50,000
Arpa-e	$ 10,000
AG2PI	$ 10,000
New Position at University of Arizona	$1,000,000
USDA NIFA $500,000	
BBSRC	
Royal Society International collaborative award (2019-2022) with IRD (France) and ISRA (Senegal)	>$250,000
BBSRC	
Breakthru: Developing Soil Compaction Resistant Wheat (2022-2025)	>$1,200,000