CHANGING WHAT'S POSSIBLE

Rhizosphere Observations Optimizing Terrestrial Sequestration (ROOTS) Program Overview

B. PROGRAM OVERVIEW

1. SUMMARY

Agricultural intensification has resulted in a ten-fold increase in crop yield over the past hundred years, but these advances have not occurred without costs: soils have eroded and soil quality has decreased, incurring a soil carbon debt equivalent to 65 ppm of atmospheric CO_2. Increased fertilizer use causes the majority of the emissions of the greenhouse gas N_2O, and drought stress increasingly threatens yields. Given the scale of domestic (and global) agriculture resources, there is great potential to reverse these trends by focusing plant breeding toward new cultivars with enhanced root systems to improve soil quality and improve biogeochemical cycling. Development of new root-focused cultivars could dramatically and economically reduce atmospheric CO2 concentrations without decreasing agricultural yields. To this end, the ARPA-E program, Rhizosphere Observations Optimizing Terrestrial Sequestration (ROOTS), is pursuing technologies that increase the precision and throughput of crop breeding for improved root-soil biogeochemical function. ROOTS seeks to develop novel, non-destructive, field deployable technologies to: (1) measure root functional properties; (2) measure soil functional properties; and (3) advance predictive and extensible models that accelerate cultivar selection and development. These technologies—especially integrated systems—could greatly increase the speed and efficacy of discovery, field translation, and deployment of improved crops and production systems that significantly improve soil carbon accumulation and storage, decrease N_2O emissions, and improve water efficiency. The aspiration of the ROOTS program is to develop crops that enable a 50% increase in carbon deposition depth and accumulation, a 50% decrease in fertilizer N_2O emissions, and a 25% increase in water productivity. Taken over the 160 million hectares of actively managed U.S. cropland, such advances could mitigate ~10% of total U.S. greenhouse gas emissions (GHG) annually over a multi-decade period, while also improving the climate resiliency of U.S. agricultural production.

2. MOTIVATION

The challenge of greenhouse gas mitigation and the potential for soil carbon storage

Carbon dioxide—the most prevalent GHG—is naturally present in the atmosphere as part of the Earth's carbon cycle (the natural circulation of carbon through the atmosphere, oceans, and terrestrial biosphere). Human activities are altering the carbon cycle—both by adding more carbon dioxide (CO_2) to the atmosphere and by influencing the ability of natural sinks, like forests, pastures and cropland, to remove CO_2 from the atmosphere. The main anthropogenic activity that emits CO_2 into the atmosphere is the combustion of fossil fuels (coal, natural gas, and oil) for energy and transportation. To avoid the predicted increases in global temperatures associated with increased atmospheric concentrations of GHGs, the U.S.—and the world—needs to drastically decrease GHG emissions and find ways to reduce the concentration of GHGs in the atmosphere.

Soils constitute the largest terrestrial organic carbon pool, estimated at 2400 petagrams of carbon (PgC), integrated from the surface to 2 m depth.[1] This is three times the amount of CO_2 currently in the atmosphere (on a C equivalent basis: ~830 PgC) and 240 times current annual fossil fuel emissions (~10 PgC/y).[2]

The primary carbon exchange between the atmosphere and the terrestrial ecosystem is the incorporation of CO_2 (~120 GT/yr^{-1}) into plant biomass through photosynthesis and the release of CO_2 from previously fixed carbon through plant and microbial respiration. A large fraction of the carbon dioxide that is captured during photosynthesis is rapidly returned to the atmosphere, and only a minor fraction, approximately 2.5 percent, enters the stable pool of soil carbon. Hence, manipulation of the soil carbon balance, by even a few percent, represents significant greenhouse gas mitigation potential.

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[1] Batjes, N. H. Total carbon and nitrogen in the soils of the world. *European journal of soil science* **47**, 151-163 (1996).

[2] Ciais, P. *et al.* Cambridge University Press, Working Group I Contribution to the Intergovernmental Panel on Climate Change Fifth Assessment Report Climate Change: The Physical Science Basis edition TF Stocker et al (2013).

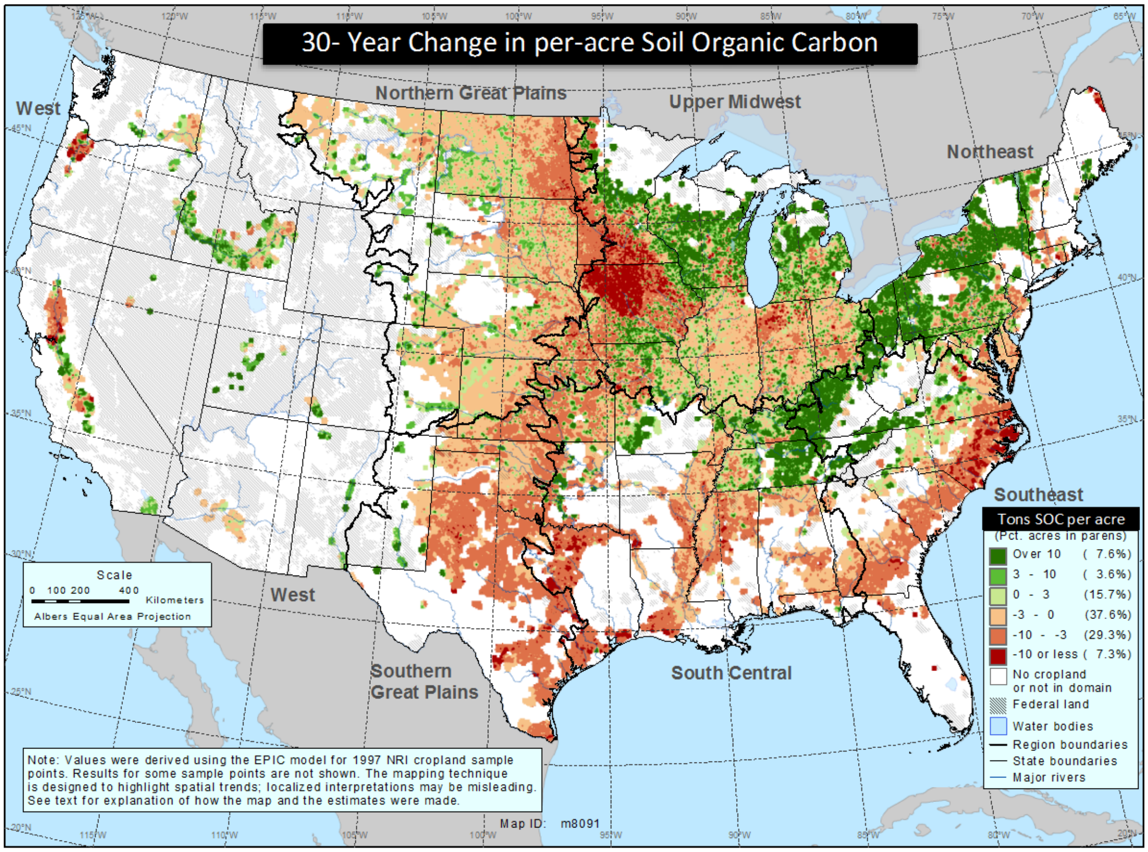

Figure 1: USDA/NRCS 2006 model simulation reporting change in tons of soil organic carbon per acre in U.S. croplands over 30 years. The total percentage of all cropland acres is shown in parentheses on the right side of the Figure.[4] The green areas on the map show increases in soil organic carbon and the red areas indicate losses.

Unfortunately, there has been considerable loss of soil organic matter (SOM) in key farming regions across the U.S. over the past several decades. The Northern and Southern Great Plains combined have lost almost four percent of soil organic carbon on a per acre basis over the last 30 years.[3] About a third of the world's soil has already been degraded—because of increasing atmospheric temperature, over-exploitation, extensive mining of soil nutrients, inappropriate tillage, poor crop management, indiscriminate use of fertilizer, and accelerated erosion. In the U.S., the SOM degradation trend is acute: a USDA/NRCS simulation of the change in soil organic carbon estimated that nearly three-fourths of the cropland acres lost soil organic carbon over 30 years, see Figure 1.[4] Moreover, losses in SOM are accompanied by real economic costs. It is estimated that the total annual cost of erosion from agriculture in the U.S. is about US$44 billion per year—$247 per hectare of cropland and pastureland.[4] On a global scale, the annual loss of more than 75 billion tons of topsoil costs the world about $400 billion per year, or approximately $70/person/year.[5]

These continuing and sustained losses of soil carbon and soil economic value provide clear motivation for soil improvement programs. Soil carbon stocks can be augmented by increasing the rate of carbon additions to the soil or by reducing the rate of decomposition of organic matter already present in the soil.[5] To varying degrees both can be achieved through a

[3] USDA, National Resource Conservation Service. Model Simulation of Soil Loss, Nutrient Loss, and Change in Soil Organic Carbon Associated with Crop Production. June 2006.

[4] Eswaran, H., Lal,R. and Reich R.F. 2001. Land degradation: an overview. In: Bridges, E.M., I.D. Hannam, L.R. Oldeman, F.W.T. Pening de Vries, S.J. Scherr, and S. Sompatpanit (eds.). Responses to Land Degradation. Proc. 2nd. International Conference on Land Degradation and Desertification, Khon Kaen, Thailand. Oxford Press, New Delhi, India.

[5] Paustian, K., Agren, G. & Bosatta, E. Modelling litter quality effects on decomposition and soil organic matter dynamics. *Driven by nature: Plant litter quality and decomposition* (1997)

variety of soil management practices.[6,7] SOM primarily enters the soil as root carbon.[8] A potential path to increases soil carbon stocks is the development of crop cultivars that input a greater quantity of carbon into the soil through their roots or grow deeper root systems, which would increase the mean residence time of deposited carbon in the soil.[9] If developed, such plants could be deployed rapidly, and at scale, due to continuous genetic turnover and active land management in agricultural croplands. Improving plants to increase soil carbon sequestration represents an untapped and economic net carbon sink with significant economic potential.

ARPA-E commissioned researchers at Colorado State University to analyze the impact of increased root depth and increased root input on soil carbon stocks. The analysis was performed using the CENTURY ecosystem biogeochemistry model[10], which is a process model that uses data on climate, soil physical properties and land management practices to estimate soil organic carbon (SOC) stock changes. Data on root depth distributions and soil depth-related controls on SOC turnover rates, were coupled to an analytical steady-state solution for SOC pools in CENTURY, to estimate SOC changes through the full soil profile as a function of changes in plant root carbon inputs. Multiple scenarios of altered crop root systems were analyzed: the quantity of carbon allocated to the roots was increased between 0%-100%, and root depth profiles were shifted between representations of relatively shallow maize root systems to representations of deep rooting grass species. The analysis covered approximately 160 million hectares of actively managed US cropland that have suitable soil types and depth. The model predicted that even modest gains in soil carbon deposition or rooting depth would provide significant offsets to U.S. GHG emissions. Therefore, a breeding platform that enables selection of plant roots with greater carbon deposition and depth is likely to provide real GHG mitigation benefits. Highly optimized root systems—those that have the largest increases in mass and depth—have the potential to increase equilibrium SOC stocks by more than 3.5 times the current content. As seen in Figure 2, annual CO_2 sequestration in a highly optimized scenario is close to 60% of U.S. transportation emissions.[11]

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[6] Paustian, K., J. Lehmann, S. Ogle, D. Reay, G.P. Robertson and P. Smith. Climate smart soils. *Nature* **532**, 49-57 (2016)

[7] Smith, P. Soil carbon sequestration and biochar as negative emission technologies. *Global Change Biology* **22**, 1315-1324 (2016)

[8] Rasse, D. P., Rumpel, C. & Dignac, M.-F. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant and Soil* **269**, 341-356 (2005)

[9] Kell, D. B. Large-scale sequestration of atmospheric carbon via plant roots in natural and agricultural ecosystems: why and how. *Phil. Trans. R. Soc. B* **367**, 1589-1597 (2012)

[10] Parton, W. J., Schimel, D. S., Cole, C. & Ojima, D. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. *Soil Science Society of America Journal* **51**, 1173-1179 (1987)
For more information: http://nrel.colostate.edu/projects/century5/reference/html/Century/overview.htm Accessed 3/30/2016

[11] Paustian, K., Campbell, N., Dorich, C., Marx, E., and Swan, A. Assessment of potential greenhouse gas mitigation from changes to crop root mass and architecture. *Report to ARPA-E. Accessible at: (*https://arpa-e-foa.energy.gov/Default.aspx#FoaId40aa63a7-689b-4307-90b2-c1b98a2148a3*)*

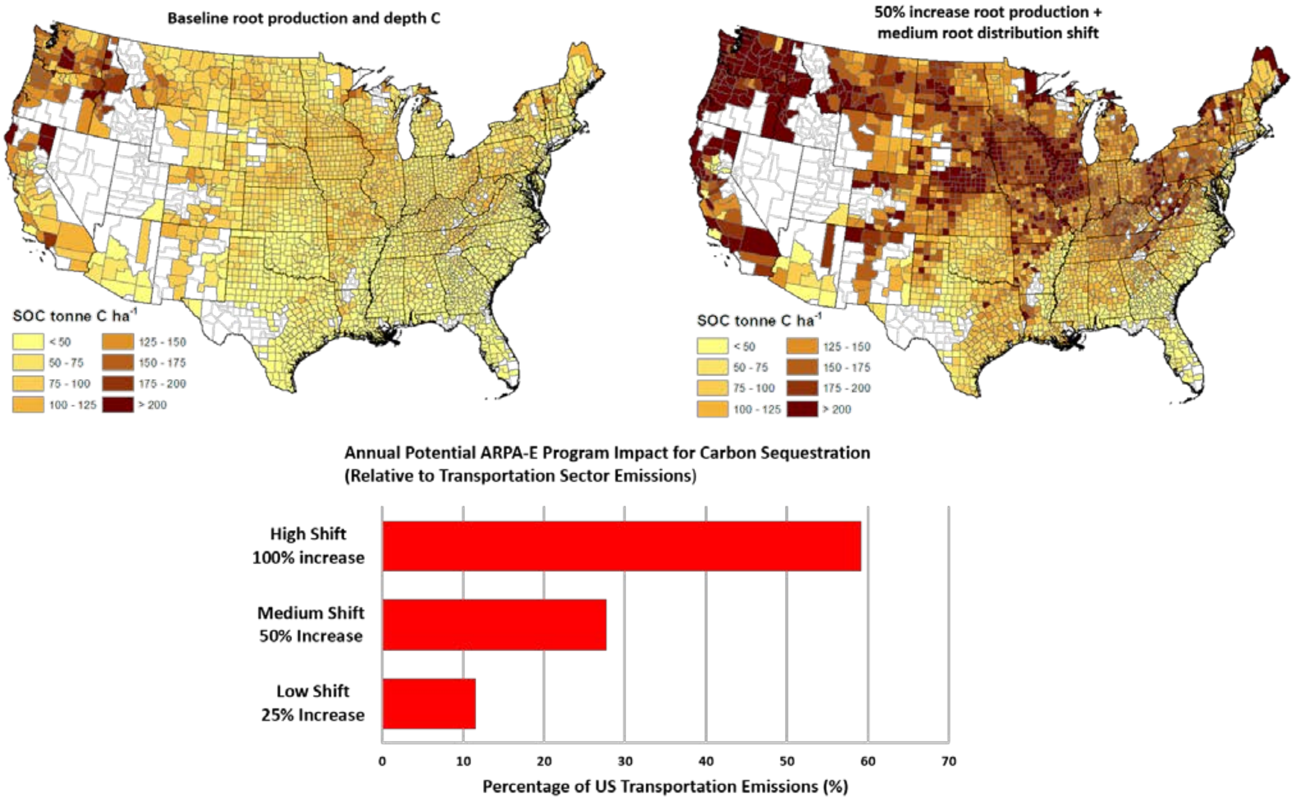

Figure 2: Geographic distribution of steady-state soil organic carbon (SOC) stocks (0-200 cm) on cropland and pasture/hay land under baseline (i.e. current) conditions and under a scenario for 50% increased root C inputs and deeper root distributions.[11] In the bar chart at the bottom, the sequestration potential of the modeled acres is the aggregation of simulations of increased root mass [+25,50,100%] and increased root depth [Low (20% of biomass shifted to next lowest root layer), Medium (annual crops shifted to grass/hay root profiles), High Shift (all crops shifted to a model root distribution)] at steady state.

Inherent Value of Soil Carbon

While industrial carbon capture methods incur significant cost and efficiency penalties, carbon captured and stored as soil organic matter is inherently valuable and enables greater agricultural efficiencies. Advanced root systems that increase SOM can improve soil structure, fertilizer use efficiency, water productivity, crop yield, climate resiliency, and limit topsoil erosion—all of which provide near-term and sustained economic value to farmers and ecological value to the public. SOM is a key component of soil quality that sustains many important soil functions by providing the energy, substrates, and biological diversity to support metabolic and physical processes that influence aggregation, infiltration, and decomposition. According to the USDA Natural Resources Conservation Service, every 1 percent of SOM can provide ~$29 per acre in the U.S. Midwest through improved nutrient and water availability. SOM helps retain water in two ways. First, SOM has higher water holding capacity compared to mineral soil, which translates into more water available to plants.[12] Second, SOM improves the soil structure and stability—porosity, water infiltration and water transport.[13] Finally, SOM supports rich communities of microbes and insects that enhance soil structure and unlock nutrients for plant growth.[14,15]

[12] Hudson BD. Soil organic matter and available water capacity. *Journal of Soil and Water Conservation* **49**, 189-94 (1994)

[13] Franzluebbers A. Water infiltration and soil structure related to organic matter and its stratification with depth. *Soil and Tillage Research* **66**, 197-205 (2002)

[14] Richardson, A. E. & Simpson, R. J. Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant physiology* **156**, 989-996 (2011)

[15] Pennsylvania State University Extension Service: http://extension.psu.edu/plants/crops/soil-management/soil-quality/earthworms Accessed 3/30/2016

Need for Increased Nitrogen Use Efficiency

Nitrogen use on U.S. agricultural and range lands is responsible for ~74% of nitrous oxide (N_2O) emissions, principally caused by fertilizer inefficiency. Expressed in CO_2 equivalents, this is 2.5% of all U.S. GHG emissions. Unlike anthropogenic CO_2 sources, N_2O is often emitted diffusely through N fertilizer oxidation. As such, prevention of N_2O emissions is likely the best method to mitigate this potent GHG. Given current efficiencies, N_2O emissions will increase as more fertilizer is used to drive higher productivity. However, as more fertilizer is applied, the fraction of fertilizer incorporated into the crop decreases. This limits crop yield and leads to substantial nitrogen leaching and reactivation to N_2O. Selection for cultivars with enhanced nitrogen capture capacity will enable greater productivity and complement the gains made by precision agriculture-enabled management changes.

The trade-off between yield and nitrogen emissions only holds for a given efficiency regime, see Figure 3. ARPA-E hopes to disrupt this relationship through improved root and root-soil function. For this reason, ARPA-E believes that increased root carbon, increased above-ground carbon and decreased N_2O emissions are fundamentally compatible and mutually reinforcing outcomes. Achieving the goal of reducing net GHG emissions requires that increased carbon storage is not offset by N_2O emissions.[16] Therefore, traits are required that improve both carbon deposition and nitrogen uptake.

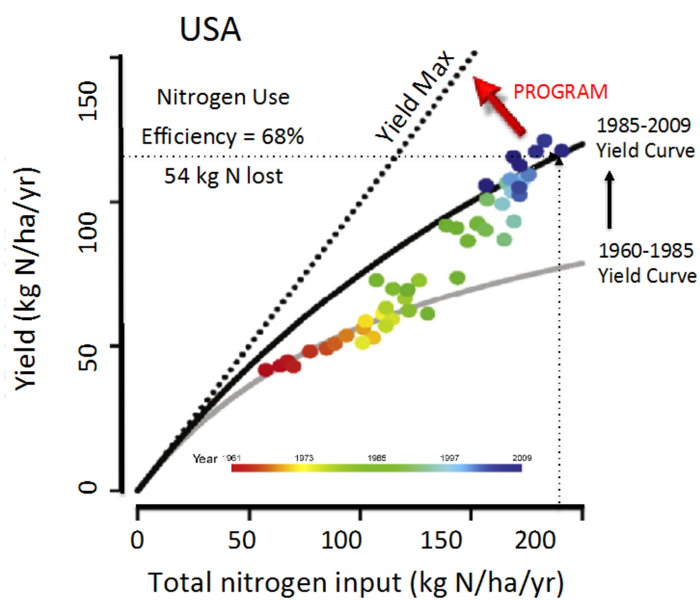

Figure 3: Historical yield of plant-nitrogen uptake as a function of nitrogen fertilizer in the U.S., demonstrating the potential for nitrogen savings in U.S. agriculture. The Yield Max indicates 100% fertilizer efficiency, while the yield curves project the maximum production per acre in a given fertilizer regime demonstrating the importance of improving nitrogen uptake to enable further yield increases[17].

Water Productivity

The impacts of drought in the United States impose significant economic costs. The economic impacts of the recent California drought, for instance, are estimated to be $2.7 billion.[18] These impacts are likely to increase as drought risks throughout the U.S. are exacerbated by the changing precipitation patterns resulting from climate change. Model projections indicate that the impact of climate change on drought frequency and severity will vary by region, with the southwestern U.S.

[16] Li, C., Frolking, S. & Butterbach-Bahl, K. Carbon Sequestration in Arable Soils is Likely to Increase Nitrous Oxide Emissions, Offsetting Reductions in Climate Radiative Forcing. *Climatic Change* **72**, 321-338 (2005)

[17] Lassaletta, L., Billen, G., Grizzetti, B., Anglade, J. & Garnier, J. 50 year trends in nitrogen use efficiency of world cropping systems: the relationship between yield and nitrogen input to cropland. *Environmental Research Letters* **9**, 105011 (2014)

[18] Richard E. Howitt, Duncan MacEwan, Josué Medellín-Azuara, Jay R. Lund, Daniel A. Sumner. Economic Analysis of the 2015 Drought for California Agriculture. Center for Watershed Sciences, University of California – Davis, Davis, CA (2015)

and Rocky Mountain states likely to experience the largest increases in drought frequency. Additionally, data suggest that climate change may increase the longevity of droughts in many regions, causing events that would otherwise be mild droughts to become severe or even extreme droughts.

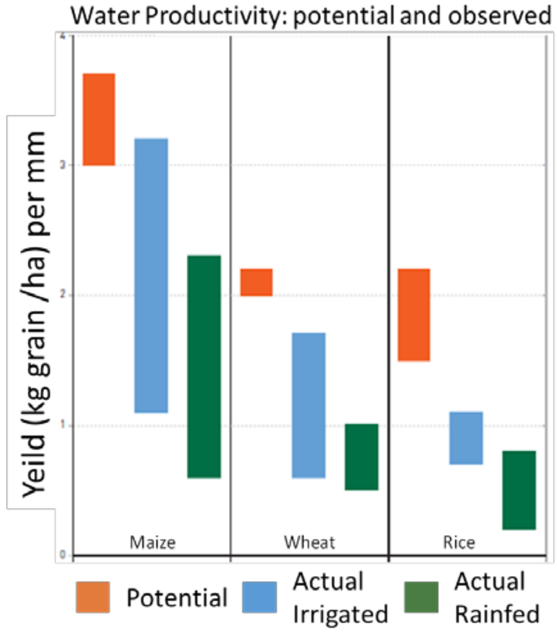

Figure 4: Water productivity gap for major irrigated and rain-fed crops[19].

As shown in Figure 4, the gap in productivity between rain-fed and irrigated systems illustrates how water can be a limiting factor to plant yield. Optimized root systems with deeper architecture are predicted to improve season-long water productivity, particularly under drought conditions.[20] In fact, drought resiliency is a key risk to meeting the future demands for food, fuel, and feed. Water productivity and drought resilience traits have the potential to mitigate the social and economic risks of systemic crop failure and help maintain high levels of agricultural feedstock production.

3. STATE OF THE ART

Root Phenotyping and Environmental Characterization

Although significant progress has been made in plant genetics and bioinformatics, a primary obstacle for continued crop improvement is in plant phenotyping, particularly phenotyping for root traits. Plant phenotypes (P) result from the complex interactions of genetics (G), environment (E), and management (M), commonly represented as P = G x E x M. Plant breeders drive crop improvement by observing component phenotypes and crossing parental lines to generate offspring with desirable combinations of traits. When possible, causal genes are identified and then the breeding progress can be accelerated and maintained by genetic screening. Modern methods such as genomic selection (GS) have the potential to drive rapid genetic gain, but creating and maintaining GS models requires high-throughput phenotypic observation. To address the gap in above-ground phenotypic data, ARPA-E is currently sponsoring a high-throughput field phenotyping program, TERRA (Transportation Energy Resources from Renewable Agriculture), focused on crop canopy attributes. As described in detail below, root phenotyping is more challenging than measuring above-ground traits. In the absence of direct observations, improvements to complex phenotypes, such as drought tolerance, are constrained because the phenotypic trait is generally controlled by multiple genes. The most desirable combinations can be found and realized much faster in breeding trials if breeders individually assess and optimize each component phenotype. Today, however, there are no high-throughput screening technologies or techniques that allow this resolution for below-ground traits.

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[19] Sadras, et al. Status of water use efficiency of main crops. *SOLAW Background Thematic Report – TR 07*, United Nations FAO (2010).
FAO. The state of the world's land and water resources for food and agriculture (SOLAW) – Managing systems at risk. Food and Agriculture Organization of the United Nations, Rome and Earthscan, London (2011).

[20] Zhan, A., Schneider, H. & Lynch, J. Reduced lateral root branching density improves drought tolerance in maize. *Plant physiology.* **168**, 1603-1615 (2015)

Current root phenotyping platforms are generally split between lab-based technologies that are high-resolution with lower throughput and poor translation to the production setting, and field-based techniques that are lower resolution, destructive, and low-throughput but that generate data more relevant to crop production and breeding.[21] Methods reflecting the current state of the art are described below.

Lab-Based Methods

Plants grown in transparent gels, against glass panes, or against transparent tubes, termed rhizotrons,[22] provide opportunities to observe roots in a manner that is non-destructive, allowing for multiple time points to be observed per plant. The concern with these systems is that the conditions do not simulate the field, and furthermore, observable roots that grow against glass may not be representative of the bulk of the root system. Improved transparent substrates have demonstrated more realistic root systems, but it is unclear how representative these systems are of the field production system.[23] These reductionist approaches are very informative, but the complex interactions of environment and management on phenotypes and gene activity confound the approach, and new methods are needed to translate lab performance to field performance to provide breeders with confidence that lab-developed genetics will perform predictably in the field.

The most technologically advanced root architecture measurements have been made with custom MRI, PET, and X-ray CT scanners. These measurements are done in pots and the plants are grown in real soils.[24] Using MRI scanners it is possible to visualize the movement of water,[25] while PET scanning allows the visualization of plant metabolites moving through the plant,[26] generating unprecedented physiological insight. The resolution of X-ray CT scanners permits visualization of soil clumps and monitoring of the roots' effects on the soil. While these techniques generate functional data useful for plant science advances, high-cost and low-throughput render them unsuitable for use in cultivar development or plant breeding. Phenome-genome linkages made in potted greenhouse samples, even if measured in natural soil, often replicate poorly in field trials.[27] These techniques face substantial challenges in deploying to field environments. For example, the resolution of MRI measurements decreases in the presence of ferromagnetic materials. Most labs remove these materials to achieve higher resolution, which limits the replicability and the range of measurement to applicable soil types.

Field-Based Methods

Many field-based methods are destructive and include soil coring and root excavation. Excavation, termed "shovel-omics," is a leading method and has been used by plant breeders for root phenotyping. Soil coring does not kill the plant per se, but is destructive to the field, and select samples may not be representative of the whole root system. The throughput and objectivity of both coring[28] and shovel-omics[29] has been greatly improved by digital analysis of the soil core or excavated root crown.[30] Applications of these technologies have made great progress in root phenotyping, but cannot be used to observe a single root at more than one point in its lifecycle. As currently practiced, these processes are manual or semi-manual, significantly limiting their throughput.

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[21] Topp, C. How Can We Harness the Quantitative Genetic Variation in Crop Root System Architecture for Agricultural Improvement? *Journal of Integrative Plant Biology*. **58**, 213-225 (2016)

[22] Rellán-Álvarez, R. *et al.* GLO-Roots: an imaging platform enabling multidimensional characterization of soil-grown root systems. *Elife* **4**, e07597 (2015)

[23] Downie, H. *et al.* Transparent soil for imaging the rhizosphere. *PLoS One* **7**, e44276 (2012)

[24] Metzner, R. *et al.* Direct comparison of MRI and X-ray CT technologies for 3D imaging of root systems in soil: potential and challenges for root trait quantification. *Plant methods* **11**, 17-28 (2015)

[25] Gruwel, M. L. In situ magnetic resonance imaging of plant roots. *Vadose Zone Journal* **13** (2014)

[26] Hubeau, M. & Steppe, K. Plant-PET Scans: In Vivo Mapping of Xylem and Phloem Functioning. *Trends in plant science* **20**, 676-685 (2015)

[27] Paez-Garcia, A. *et al.* Root Traits and Phenotyping Strategies for Plant Improvement. *Plants* **4**, 334-355 (2015)

[28] Wasson, A., Bischof, L., Zwart, A. & Watt, M. A portable fluorescence spectroscopy imaging system for automated root phenotyping in soil cores in the field. *Journal of experimental botany* **67**, 1033-1043 (2016)

[29] Trachsel, S., Kaeppler, S. M., Brown, K. M. & Lynch, J. P. Shovelomics: high throughput phenotyping of maize (Zea mays L.) root architecture in the field. *Plant and Soil* **341**, 75-87 (2011)

[30] Bucksch, A. *et al.* Image-based high-throughput field phenotyping of crop roots. *Plant Physiology* **166**, 470-486 (2014)

Other techniques allow researchers to obtain data about roots throughout the plant life cycle, but only over a fraction of the spatial extent of the root system. Field based rhizotrons[31,32] are clear plastic tubes that are placed at the time of planting and left in place as the root system develops around them. Cameras are placed down the tubes and provide very high resolution images of the limited parts of the root system that grow near the tube. These techniques have been very useful for determining numbers of root classes and growth rates but are limited by the quantity of roots that associate with the tube, concern that the tube influences the phenotypes, and general applicability to broad-scale field breeding populations. Ground penetrating radar (GPR)[33] provides relatively low-resolution images that can be used to quantify biomass and have reached resolution that is sufficient to view tuberous crops such as potato and cassava. However, it requires significant improvement to meet the needs of cost, throughput and resolution on fibrous rooted row crops, particularly when used in electrically polarizable soils.

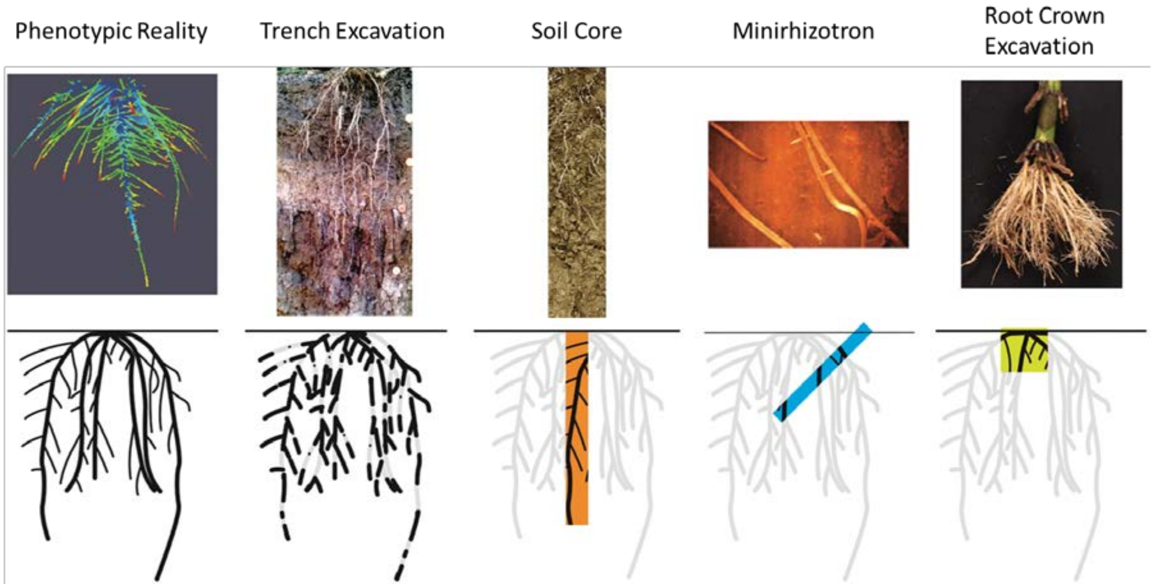

Figure 5: Current field tools for root phenotyping are low-throughput, and most are destructive and allow only partial measurement of root architecture.[21]

Sensing Soil Properties

In addition to measuring roots in the soil, there is much work done to measure the distribution of nutrients and water in the soil and to quantify the physical properties of the soil. The ability to measure plant effects on nutrients and water has been used to provide indirect trait determination,[34] and there is potential to tune imaging technologies based on soil properties to improve resolution. Current systems include nutrient and water sensors.[35,36.] These systems of sensors can be introduced into a field and provide a farmer information with respect to the most efficient application of fertilizer and water. More recent sensors under development include those that leverage microelectromechanical systems (MEMS) to provide information on the water content of the soil.[37] Advanced soil sensors, when integrated with plant functional phenotyping, may allow selection of germplasm suited to specific real-world environmental conditions.

Understanding the flux of gasses in and out of the soil could provide significant benefits to cultivar development and precision crop management. Current systems, like eddy covariance, can measure gas fluxes, but are expensive and cover a limited amount of land relative to the country's agricultural footprint. In order to better understand and screen for plant and soil

[31] Gray, S. B. *et al.* Minirhizotron imaging reveals that nodulation of field-grown soybean is enhanced by free-air CO_2 enrichment only when combined with drought stress. *Functional Plant Biology* **40**, 137-147 (2013)

[32] Iversen, C. M. *et al.*, Advancing the use of minirhizotrons in wetlands. *Plant and Soil* **352**, 23-39 (2012)

[33] Thompson, S. M. *et al.*, https://dl.sciencesocieties.org/publications/meetings/download/pdf/2013am/78536, accessed 3/30/2016

[34] Vadez, V. et al. LeasyScan: a novel concept combining 3D imaging and lysimetry for high-throughput phenotyping of traits controlling plant water budget. *Journal of Experimental Botany* (2015).

[35] Aquaspy: http://www.aquaspy.com/ Accessed 3/30/2016

[36] Trimble: http://www.trimble.com/Agriculture/sis.aspx Accessed 3-30/2016

[37] Cornell University News: http://news.cornell.edu/stories/2013/10/new-micro-water-sensor-can-aid-growers Accessed 3/30/2016

properties, cheaper and distributed sensors that measure CO_2, N_2O, and water vapor, among other gasses, are needed. An appendix is included at the end of this document to provide additional background information on soil and root properties.

Survey of Additional Technologies

The problems of imaging through complex media are similar to challenges faced by the medical, aerospace, mining, oil exploration, and defense industries.[38,39] Several classes of novel sensors and imaging platforms may be adapted to the tasks of root phenotyping. One example is low-field magnetic resonance imaging (MRI), which limits risks arising from ferromagnetic materials in soil.[40] Thermoacoustic imaging has demonstrated promising preliminary results in highly dispersive media.[41] Other examples include nuclear quadrupole resonance and X-ray computed tomography with sophisticated reconstruction algorithms. In addition to the potential to "see" through the soil, innovative robotics may deliver sensors by coupling small profile mobile probes[42] to a range of analytical techniques that can be implemented in extremely low profile endoscopic configurations[43]. Sensor packages may include photoacoustics, fluorescence, and coherent anti-Stokes Raman spectroscopy, among others, and have the potential for a disruptive increase in capability over state of the art. A partial survey of existing and experimental technologies is shown in Figure 6. These are representative examples only, and are not intended to limit the range of technologies proposed in response to this FOA.

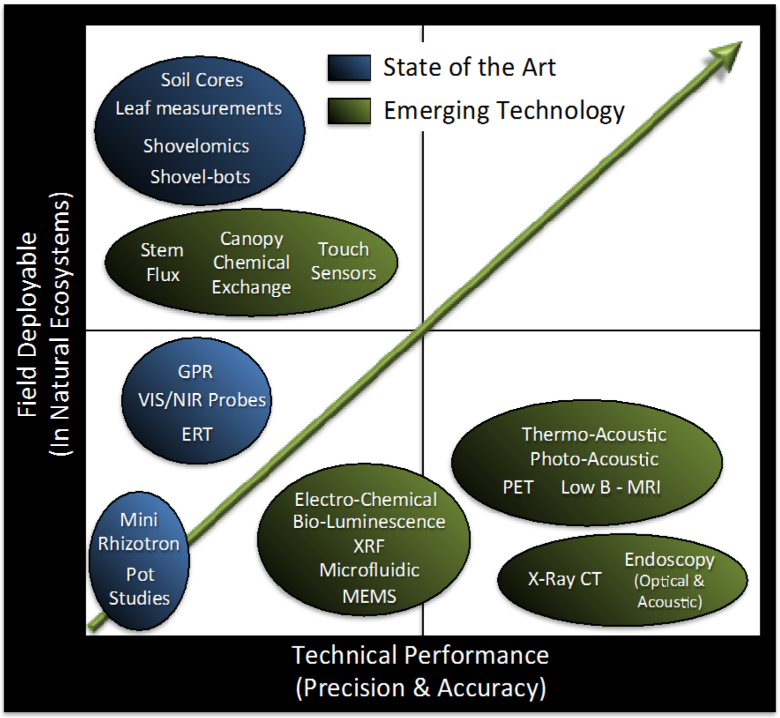

Figure 6: Survey of tools classified by qualitative measures of field deployability and technical performance. Tools of interest are not limited to those listed, and may include sophisticated above-ground sensors, tools that provide information about the flux of nutrients to the root system, sensors that provide information about the soil and the nutrients in it, or sensing/imaging tools that directly probe plant roots. (ERT – Electrical Resistivity

[38] MacDonald, J., Lockwood, J. Alternatives for Landmine Detection http://www.rand.org/pubs/monograph_reports/MR1608.html, Accessed 3/30/16

[39] Shell Gamechanger, MRI. http://www.shell.com/energy-and-innovation/innovating-together/shell-gamechanger.html, Accessed 3/30/16

[40] Sarracanie, M. et al. Low-Cost High-Performance MRI. *Scientific Reports* **5**, 15177 (2015)

[41] Nan, H. *et al.* Non-contact thermoacoustic detection of embedded targets using airborne-capacitive micromachined ultrasonic transducers. *Applied Physics Letters* **106**, 084101 (2015)

[42] Tully, S. & Choset, H. A Filtering Approach for Image-Guided Surgery with a Highly Articulated Surgical Snake Robot. *IEEE Transactions on Biomedical Engineering* **63**, 392-402 (2015)

[43] Seibel, E. J. *et al.* in *SPIE BiOS.* 82180B-82180B-82189 (International Society for Optics and Photonics)

TomographyPET – Positron Emission Tomography, XRF – X-Ray Fluorescence, GPR – Ground Penetrating Radar, MEMS – Micro-Electromechanical Systems, CT – Computed Tomography).

Root-Soil Modeling

Design, discovery, and development of traits with high heritability requires high-throughput measurement of functionally important plant phenotypes (e.g., physiology) and environmental (*e.g.*, soil) characteristics. Modeling represents an excellent opportunity to determine characteristics that are costly to measure, and improve them faster, by establishing correlations to cheaper-to-measure features. For example, it may be possible to estimate and improve the fine-root structure of fine roots in deep soil by making soil density surveys and combining them with above-ground physiological or morphological measurements, or to determine correlations between features present in early stages of development with those determinable at the end of the growing season. Such models would reduce the cost of sensor data needed to validate a new root ideotype design or screen for a phenotype in field populations. Multiple root models have been created and have already shown success for trait improvement.[44] For example, a mechanistic model has been used to predict root system water efficiency (a physiological phenotype) by optimizing a lateral root branching trait[45] (an architectural phenotype). This prediction was then validated by testing recombinant inbred lines with divergent phenotype values for the later root branching trait, and thereby demonstrated a wide range of grain yield under drought conditions. Given this validation, this trait became a strong candidate to introduce into elite cultivars to improve their drought tolerance.

C. PROGRAM VISION

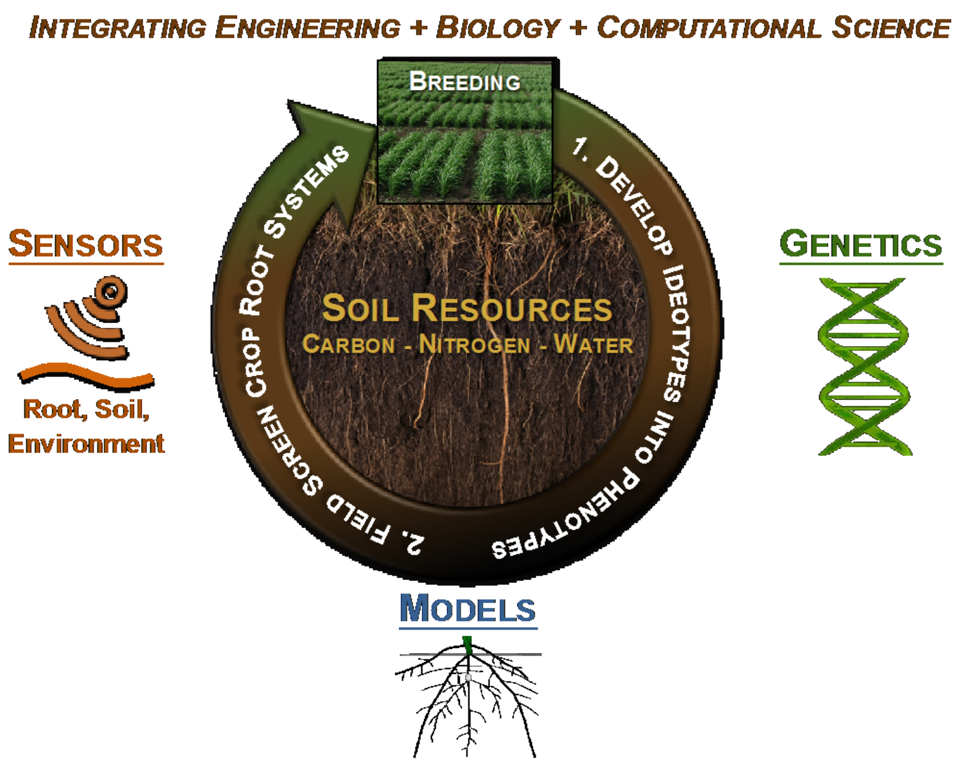

Figure 7: Program Vision - Breeding for Enhanced Soil Quality, Crop Productivity and Greenhouse Gas Mitigation

Precision phenotyping of roots and their interactions with soils under natural field conditions is a complex, system-level challenge that requires the integration of multiple scientific and engineering disciplines. ARPA-E encourages interdisciplinary teams that will improve crop breeding with sensing technology and mechanistic modeling. This funding

[44] Warren, J. M. *et al.* Root structural and functional dynamics in terrestrial biosphere models–evaluation and recommendations. *New Phytologist* **205**, 59-78 (2015)

[45] Zhan, A., Schneider, H. & Lynch, J. Reduced lateral root branching density improves drought tolerance in maize. *Plant physiology* **168**, 1603-1615 (2015)

opportunity should create integrated systems that enable crop genetic improvement of root-soil functional traits that increase soil organic carbon, increase fertilizer efficiency, decrease N_2O emissions, and increase water productivity. The key technical challenges ROOTS aims to solve are: low-throughput for field screening; poor phenotypic correlation of traits measured in controlled environments to field environments; and lack of systematic integration of roots, shoot, and soil properties in the process of ideotype design and development. Ideal systems should include substantial technical development across some, or all, of the following areas: tools for root phenotyping; tools for soil functional characterization; modeling that helps make linkages between environmental, phenomic, and genomic variation that are relevant to breeding; and identification and integration of phenotypes-into-cultivars.

By program completion, performers will be expected to demonstrate that these systems can select for these traits in field conditions for either, or both, (1) ideotype identification and translation and (2) field cultivar selection, as shown in Figure 7. Submissions that focus strictly on sensor tool development will be considered for proof-of-concept demonstrations. Submissions that leverage above-ground tools to infer below-ground characteristics are of definite interest, but any sensor development must be technologically distinct from those developed through ARPA-E's TERRA program. All submissions should describe how their project will drive large-scale adoption of agricultural systems that enable carbon sequestration and/or improved agricultural water and nitrogen use.

D. TECHNICAL CATEGORIES AND COMPONENTS

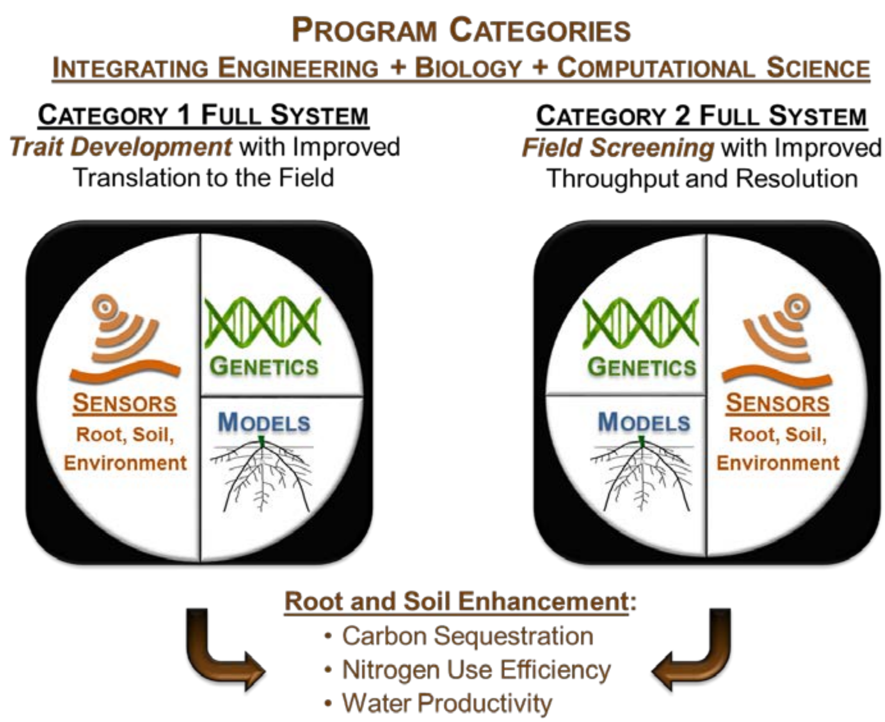

Figure 8: Program Categories - Trait Development and Field Screening

This program will be divided into two functional categories set up to address the fundamental problems of root phenotyping today, as shown in Figure 8. Category 1 projects should address the challenge of poor translation of high quality phenotyping platforms and observations to field sites, by designing and field-validating new root-soil ideotypes. Category 2 projects should address the need for high-throughput and resolution of root and soil screening technologies available to breeders, by demonstrating field deployability of systems for screening cultivars. Teams are encouraged to address both categories in an integrated submission. All submissions (Category 1, Category 2 or combined Category 1 and 2) must explain how the project will address the broader biogeochemical goals of the program: increases soil organic matter, particularly through deeper and increased annual flux of carbon into the soil; decrease in N_2O emissions, particularly through decreasing fertilizer requirements; and improved tolerance of crops to drought conditions, possibly by long term improvement of soil properties such as water holding capacity.

Regardless of which functional categories are addressed, submissions must either discuss the three Components immediately following (i.e., Component A (Sensors), Component B (Models), and Component C (Genetics and Environment)), or Component A exclusively. The latter will be considered for awards of shorter duration and smaller amounts supporting proof-of-concept demonstrations. **ARPA-E's preference is for submissions that address all three Components.**

Component A (Sensors): Advanced sensors and imaging technology for characterization of roots and soils. The terms "sensors" and "imaging technology" *are meant to be broadly interpreted* as referring to any method of measurement (direct or indirect) with breeding relevance. Submissions should explain their strategy for moving sensors from proof-of-principle to in-field and also for automatically collecting, analyzing, and reducing their data.

Component B (Models): Predictive and extensible models of plants and soils to accelerate root breeding programs. Models that predict how traits will react to novel conditions or which traits are desirable in a given geography could limit the number of field trials that are needed to advance a new cultivar. Modeling may also be used during measurements by guiding to sensors toward areas most likely to be informative.

Component C (Genetics and Environment): Genetic resources and characterization of germplasm performance in multiple environments and/or management regimes for phenotypes that address ROOTS biogeochemical goals. Submissions should justify the specific phenotypes or soil characteristics targeted. While ARPA-E expects novel sensors developed in a project should be integrated into a projects' genetic strategy, projects may initially utilize pre-existing technology.

CATEGORY 1: IDEOTYPE DEVELOPMENT PLATFORMS – IDENTIFICATION OF PHENOTYPES AND THEIR CAUSAL GENES FOR IMPROVEMENTS IN ROOT SYSTEM FUNCTION

Current crops have been designed for high shoot yield and agronomic value, with below-ground biogeochemical function optimized only due to correlation with yield. ARPA-E believes that explicit design of root ideotypes can improve root-soil biogeochemical function and still maintain high yields. The goal of Category 1 projects is a validated root ideotype design and the development of tools for root ideotype design. A project should also identify genetic markers for this ideotype as well environmental (*e.g.*, soil) characteristics highly correlated with phenotypic expression. Finally, these ideotypes should be validated in a representative range of field environments (*e.g.*, multiple soil types) with high correlation to predictions from tests done in a small number of fields or in a controlled environment.

Category 1 sensors should have sufficient resolution for phenotype identification and sufficient throughput for genetic marker identification. If controlled environments / greenhouses are proposed, the applicants must justify their relevance to field conditions and explain their plan for in-field phenotype verification.

Category 1 models should directly support the identification of new root ideotypes, the identification of genetic markers or causal genes, and improve the success rate of field validation trials. The models should be designed to incorporate findings from the novel sensor method and help determine the best field implementation of the sensor methods. Models that help relate diverse measureable characteristics of root-soil systems, such as root architecture and root physiology, to one another are of particular interest.

Category 1 genetics and environment components should achieve field validation of new phenotypes or identification of genetic markers or causal genes. These genetic markers or key phenotypes could then be transitioned to higher throughput sensors in Category 2 field screening programs to mobilize traits in production settings.

Outcomes of successful projects in this category could be genetic improvement of a carbon sequestration trait by breeding, transgenes, or gene editing methods; development or refinement of a predictive model to identify phenotypes that increase nutrient acquisition efficiency or root biomass; development of a field proxy for a phenotype easily measured in controlled environments; and/or methods that identify phenotypes under high degree of genetic control that require a smaller number of plants and/or predict the impact of field environment variation on trait expression.

CATEGORY 2: FIELD SCREENING PLATFORMS – IDENTIFICATION OF PLANTS IN THE FIELD THAT EXHIBIT DESIRED PHENOTYPES VIA HIGH-THROUGHPUT AND MINIMALLY DESTRUCTIVE METHODS

ARPA-E seeks to fund development and validation of systems of sensors and models that enable high-throughput field phenotyping for significant biogeochemical traits. Applicants are expected to provide details on the phenotypes they will characterize and may utilize known varieties with increased rooting depth or other target traits to validate their systems. Applicants should describe details of field studies and collection plans for ground truth and calibration data.

Category 2 sensors should be field deployable in breeding conditions, minimally destructive, and high-throughput. It is expected that sensors developed in Category 2 will generally be lower resolution but higher throughput than those developed in Category 1. For example, aggregate measurements of root mass and structure are expected to be well-suited for Category 2, while measurements of fine root structure are anticipated to be better suited for Category 1.

Category 2 models should accelerate the process of field screening and address the throughput and resolution limitations expected of these sensors. For example, these models may predict below-ground phenotypes from near-surface or above-ground phenotypes and, by establishing these correlations rapidly, promote or discard individual lines. Integration of soil and root modeling might reduce the frequency of measurements needed for accurate prediction of field performance of breeding material.

Category 2 genetic outcomes would be the ability to select individuals with improved root-soil functional characteristics. Category 2 environmental variation should account for a representative range of soil variation, relevant to a significant fraction of the U.S. commercial range of the chosen crop. An example of a potential technology for this category could be the development of a thermoacoustic measurement platform capable of passing over field plots and imaging hundreds of plants per day. A team could utilize this tool on a population of wild accessions originating from drought prone environments over multiple growing seasons to identify a quantitative trait locus (QTL)[46] linked to increased root proliferation.

DUAL CATEGORY SUBMISSIONS

Efforts that link approaches and provide continuity to the process would be highly beneficial, and applicants should not feel constrained to tailor their concepts to fit a specific category, particularly where an Applicant's sensor technology may be applicable to both categories. Certain sensor technologies may be usable for both categories by altering how they were deployed. For example, a Category 1 implementation of magnetic resonance imaging of roots might use a longer averaging time than a Category 2 implementation, or it might involve a soil invasive element in Category 1 and be only used on the surface for Category 2. A dual category project might screen, in its Category 2 element, for the markers identified in its Category 1 element.

E. TECHNICAL PERFORMANCE TARGETS

Table 1: Category 1 Metrics

Category 1 Ideotypes into Phenotypes		
ID	Description	Target
Component A – Sensors		
1A.1	Instrumentation Target	CV < 5% for identification or root or soil characterization R^2 >.75 ground truth value

[46] A quantitative trait locus is a specific region of DNA in an organism's genome that is statistically correlated with an observed phenotype. Multiple QTLs can be identified throughout a genome to characterize complex, multi-gene traits. Miles, C. & Wayne, M. Quantitative trait locus (QTL) analysis. *Nature Education* **1,** 208-216 (2008)

1A.2	Technical Repeatability	>95%
1A.3	Throughput / Scale	>500 plants, 3 times per season, in translatable conditions
Component B – Models		
1B.1	Improve Throughput	Allows 10-fold reduction in the number of plants to screen for phenotype identification
1B.2	Enhance Translation	Enable correlations between measured values and field performance with $R^2 > 0.6$
Component C - Genetics and Environment		
1C.1	Genetic Basis of Root Traits	Target traits with heritability: > 0.5 *OR* Identify 3 causal genes or linked markers that predict >50% of genetic component of a trait
1C.2	Genetic (G) and Environment (E) Interaction	Quantify GxE influence on traits by measurement in at least 3 environments
1C.3	Quantify Impact	Ideotypes achieve >25% improvement of carbon sequestration, nitrous oxide reduction, or water productivity validated either with field measurement and/or model.

Supplemental Explanation of Category 1 Technical Targets:

- All criteria are under like environmental conditions and best land management practices.
- All genetic improvements must be yield neutral or yield positive, once germplasm is re-optimized.

1A.1 Target refers to the CV and R^2 for the chosen soil or phenotype from Table 3. Sensor metrics are specified in Table 3.
R^2 is defined as the sample coefficient of determination, which represents the proportion of the variation of the data in question as explained by the regression, and coefficient of variation (CV) is defined as the root mean squared error, divided by the *y*-value for the data point, expressed as a percentage.[47]
Ground truth is defined as relative to state of the art in measuring the indicated property.

1A.2 Technical Repeatability is defined as precision under repeatability conditions, where repeatability conditions are defined as conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time. Definition from ISO 5725-1:1994.

1B.2 Correlation, as quantified by the correlation coefficient, measures the strength of a relationship between two variables.[48]
1C.1 Heritability is a measure of the phenotypic variation of a population observed in an environment that is due to genetic variation within the population. Broad sense heritability can be represented by the ratio $H^2 = Var(G)/Var(P)$. [48]

[47] Chapters 11 and 12 of Walpole, Myers, Myers, Ye. Probability and Statistics for Engineers and Scientists. 8th edition. Pearson Education International. 2007.
[48] Principles of Population Genetics, 4th Ed. Hartl and Clark, 2007

Table 2: Category 2 Metrics

Category 2 Field Screening Crop Root Systems		
Component A – Sensors		
2A.1	Instrumentation Target	Instrumentation Target: CV< 10% of root or soil property R^2 >.6 ground truth value
2A.2	Technical Repeatability	>90%
2A.3	Throughput / Coverage	2 hectares with 2000 plant accessions each measured 3 times during growing season
Component B – Models		
2B.1	Improve Throughput	25%-50% improvement of throughput in field breeding.
Component C - Genetics and Environment		
2C.1	Genetic Basis of Root Traits	Target traits with heritability: > 0.4, or establish predictive models (e.g. Genomic Selection) accounting for >50% of heritable variation
2C.2	Genetic (G) and Environment (E) Interaction	Quantification of GxE influence on cultivar, by measurement in at least 3 environments with maximum coverage of relevant commercial crop growth
2C.3	Quantify Impact	Cultivar with wide deployment that achieves >25% improvement of carbon sequestration, nitrous oxide reduction, or water productivity validated either with field measurement and/or model.

Supplemental Explanation of Category 2 Technical Targets:

- All criteria are under like environmental conditions and best land management practices.
- All genetic improvements must be yield neutral or yield positive, once germplasm is re-optimized.

2A.1 Target refers to the CV and R^2 for the chosen soil or phenotype from Table 3. Sensor metrics are specified in Table 3.
R^2 is defined as the sample coefficient of determination, which represents the proportion of the variation of the data in question as explained by the regression, and CV is defined as the coefficient of variation is defined as the root mean squared error, divided by the *y*-value for the data point, expressed as a percentage.[48]
Ground truth is defined as relative to state of the art in measuring the indicated property.

2A.2 Technical Repeatability is defined as precision under repeatability conditions, where repeatability conditions are defined as conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time. Definition from ISO 5725-1:1994.

2B.1 Throughput increased is defined by a decrease the breeding cycle time or the number of required plots and locations to discriminate breeding population performance.

2B.2 Correlation, as quantified by the correlation coefficient, measures the strength of a relationship between two variables.[48]

2C.1 Heritability is a measure of the phenotypic variation of a population observed in an environment that is due to genetic variation within the population. Broad sense heritability can be represented by the ratio $H^2 = Var(G)/Var(P)$.[49]

Additional Requirements

All projects—Category 1 and/or Category 2—are expected to demonstrate commercial utility via:

(1) Crop choice of an annual or perennial crop that has a robust fine root system, well-characterized genetic resources, a sequenced genome, and access to existing breeding pipelines with commercial potential. If perennial crops are chosen, proposer must have access to established crop sites in multiple environments. Sensors with broad crop applicability are encouraged.

(2) Development of technology capable of achieving cost targets and throughput levels (at full deployment) relevant to commercial breeding.

Table 3 includes a list of particularly interesting phenotypes and soil characteristics, and metrics for measuring those phenotypes. All submissions must address at least one of the listed phenotypes or soil characteristics. Submissions with only Component A must develop a sensor capable of achieving the corresponding metrics. Submissions with all three Components are recommended to address a sensor metric in Table 3, but may argue for a different metric if applicants' proposed sensor technology is not well described by the sensor metrics below; or applicants are combining sensors and/or models that can achieve program goals without meeting the specific metrics below.

In addition to requirements in Table 3, novel sensors must be at least as accurate as the corresponding state of the art, to which they should be compared to ground-truth during the project.

Table 3: Phenotypes and Sensor Metrics

Phenotypes	Sensor Metrics
Carbon Flux and Nitrogen Flux Characteristics	
Root or Microbe Mass	Precision and repeatability within 10% on total mass of roots or microbial community. Alternatively, the team can provide 10% precision and repeatability relative to another quantity, such as soil mass or volumes. Methods capable of distinguishing root mass from residue are of interest.
Photosynthate or Exudate Flux	10% precision and repeatability on total photosynthate or exudate flux, or per a defined mass of soil.
C:N Ratio Or Lignin:Cellulose Ratio	10% precision and repeatability for C:N ratio or lignin:cellulose ratio.

Root Spatial Characteristics	
Root System Architecture	Must show significant improvement relative to state of the art in identification of root system architecture, including differentiation among roots, soil and plant litter. Examples of parameters relevant to root system architectures may include root angle, branching, depth, surface area, or length.
Root Physiology / Growth Rate	Must show significant improvement relative to state of the art. Obtaining information about carbon partitioning or composition may be considered synergistic.
Root Morphology / Anatomy	Must show significant improvement in ability to measure aspects of root anatomy, such as root hairs, rhizosheaths, or root cortical arenchyma, relative to the state of the art.
Soil Characteristics	
Bulk Density	Precision and repeatability < 3%
Nitrate Concentration	Precision and repeatability of 2 ppm.
Soil Carbon Content	Specify 0.1% precision on total soil mass or volume over an area of 10 m^2
Nitrous Oxide Concentration	Specify 10% precision over an area of 10 m^2, integrated on a weekly basis
Soil Porosity (Compaction)	Precision and Repeatability < 3% over proposed soil volume.
Soil Water Content (including water holding capacity and plant-available water)	Provide soil water content at a spatial resolution of 10 cm of depth. Precision and Repeatability < 3% over proposed soil volume.
Soil Respiration Rate	Precision and repeatability < 10% over a time interval of one day.
Soil Water Potential	Provide soil water potential at a spatial resolution of 10 cm of depth. Precision and Repeatability < 3%.
Fraction of Nitrogen Microbially-Fixed	Specify 10% precision over an area of 10 m^2, integrated on a weekly basis