Statement of Research — Larry M. York

With better root systems we can achieve sustainable yields using less fertilizer, which would increase food security, decrease atmospheric and water pollution associated with chemical fertilization, and save farmers money. While roots have long been known to be central to soil resource acquisition, relatively little is known about how specific root properties affect nutrient and water capture. In order to address this knowledge gap, I envision the emergence of **root functional phenomics** which uses high-throughput phenotyping of phenotypically diverse populations to link root form and physiology to genetics and functional utility. Over the past decade, I have continuously innovated through the development of image-based root crown phenotyping, applying advanced data analytics, combining simulation and empirical work, and studying ion uptake kinetics by roots. My work lays the ground work for ambitious projects to leverage modern technologies to better understand the functioning of root systems and to apply this knowledge to breeding efforts.

PhD Dissertation: Integration of root phenes for nitrogen acquisition in maize

Conceptually, I clarified the use of ambiguous terminology by promoting the use of phene (a term that is almost 100 years old) as an elemental unit of phenotype rather than 'trait,' categorizing root phenes based on their effects on root foraging and plant metabolism, and proposing mechanisms by which root phenes integrate within the contexts of acquiring different soil resources (York *et al*., 2013). My empirical work focused on the influence of root phene variation in maize on nitrogen acquisition in the field. I worked with DuPont Pioneer to gain access to 16 maize varieties representing the diversity of maize germplasm planted in the USA over the past 100 years and documented **evolution of root system architecture and anatomy** that corresponded to increased nitrogen acquisition efficiency at different densities and nitrogen levels, which was corroborated with simulation modeling using *SimRoot* (York *et al*., 2015). I developed an **intensive root phenotyping platform** that quantified maize root phenes among all whorls of the nodal root system for the first time and demonstrated the additive integration of phenes like nodal root growth angle and number, and lateral root branching and length that resulted in enhanced growth in low nitrogen soil (York and Lynch, 2015). My contributions to next-generation phenotyping of root systems using digital imaging have led to the development of custom programs for **automatic image analysis** that are freely available to the community (Bucksch *et al*., 2014; Colombi *et al*., 2015). Nitrate uptake kinetics determine the maximum uptake rate and ability to acquire nitrate at low concentrations. I discovered variation among maize root classes for uptake kinetics and demonstrated that **improving uptake kinetics** of lateral roots would have the greatest benefits for plant performance using simulation modeling (York et al., 2016a). I also contributed to work on how root system phenes affect intercropping of maize, common bean, and squash by developing a real-time PCR technique for quantifying the proportion of crop species' roots in mixed samples (Zhang *et al*., 2014). This dissertation research was conducted in China, South Africa, and the United States and included collaborators from Europe.

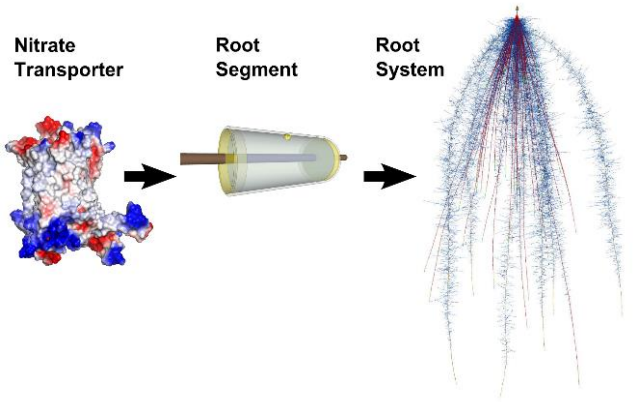


Figure 1. Nitrate uptake scales across levels

Postdoctoral Research: X-ray CT, the holistic rhizosphere, and intensive phenotyping of wheat and soybean

My work at the University of Nottingham included phenotyping the root systems of a double haploid wheat population in relation to water use in the field and using X-ray CT to phenotype recombinant chromosome substitution lines of barley, both of which are expected to contribute to genetic maps of loci affecting RSA and identification of phenes affecting resource acquisition. We are bridging the gap between phenotyping and simulation modelling by directly **importing 3D root systems from our X-ray scanning to *SimRoot*** and simulating nutrient uptake. I developed an intensive root crown phenotyping platform for wheat that not only measures root architectural phenes of the whole crown, but also of the main shoot and tiller root systems

Figure 2. X-ray CT reveals in situ root system architecture

independently for the first time. The manuscript being prepared demonstrates that most 'angles' being measured in wheat are determined by the number of tillers and that the root phenes of the main shoot are most heritable. In Nottingham, I continued my conceptual explorations in a recent manuscript on the **holistic rhizosphere** (York et al., 2016b) that provides a framework for integrating zones of the rhizosphere.

Currently, I am continuing my work at the University of Missouri working on a bespoke X-ray CT instrument. Most CT systems are commercial units and require proprietary software for reconstruction of 3D volumes. We have built CT instrument, and are implementing reconstruction in open source software. These advances could **revolutionize root X-ray scanning** by making the system 20-fold cheaper than commercial while simultaneously increasing the breadth of possible measurements. I continue innovation of high-throughput root crown phenotyping by developing a multiple camera imaging platform for soybean. Manuscripts are in preparation for this imaging platform and multiple sites and years of manual root crown phenotyping for QTL and GWAS analysis.

Future Research: Root functional phenomics of forage and field crops

My future work will be a synthesis of past work that more effectively utilizes available resources to make fundamental discoveries about root biology while supplying phenes and genes to plant breeding programs. My theory of the integration of root phenes implies that root system properties cannot be properly understood in isolation, but rather must be understood in the overall phenotypic context. Thus, I will endeavor to characterize the most multidimensional phenotypes possible, including root system architecture, anatomy, and physiology. These data alone are not sufficient to demonstrate functional significance, and because roots cannot be understood without understanding whole plant integration, I plan to measure shoot carbon balances, nutrient composition, and morphology to the greatest extent possible. The overall research paradigm of my future work is the functional phenomics pipeline (illustrated below). I will apply this pipeline to tall fescue, alfalfa, wheat, maize, and soybean which represent the most important forage and field crops in Oklahoma.

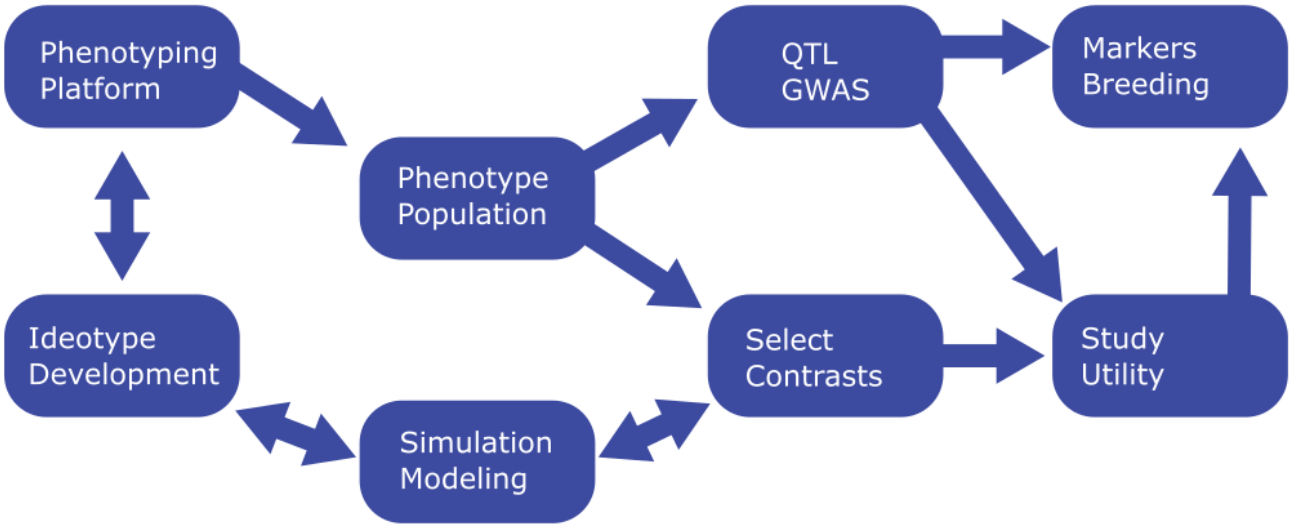


Figure 3. The functional phenomics pipeline integrates theory, field phenotyping, genetic analysis, simulation modelling, and plant physiology to supply phenes and genes for breeding.

Optimization of plant growth and architecture requires substantial balancing of resource partitioning and foraging behavior. While more roots certainly lead to quicker soil resource depletion, roots also have a substantial cost. The spatial location of soil foraging largely depends on root system architecture, starting with the numbers and angles of the axial roots, commonly the primary, seminal, and nodal roots in monocots and the primary root and first order laterals in dicots. This is the scaffolding on which root system function is built upon, and determines the broad horizontal and vertical *extent* of root foraging. However, it is the degree of lateral root branching from these axial roots that determines the *intensity* of foraging. In general, we want both the extent and intensity of root foraging to be balanced against the relative costs. Thus, the marginal value theorem from economics is a central tenant of plant physiology. In general, we can say a plant should produce more roots until the marginal benefit of growing an increment equals the marginal costs of building and maintaining that increment. This is why measuring the functional outcome of variation in root forms is imperative as it aggregates the outcomes of the multitude of balancing acts. While root system architecture largely determines these foraging aspects, it is anatomical phenes that largely determine the costs of constructing and maintaining roots. Root architectural and anatomical phenes cannot be understood in isolation, and so my future research will measure both intensively in all root samples.

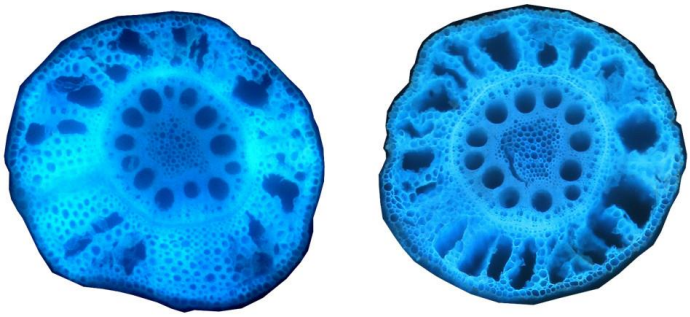
In alfalfa, I propose to use the Chilean BC_1 ($CHBC_1$) and Wisfal BC_1 ($WFBC_1$) mapping populations that have already been shown to have substantial variation in drought tolerance that could be related to root system architecture. The target tall fescue population will be one resulting from the cross of the Continental and Mediterranean morphotypes for which maps have recently been created. The nearly 300 wheat germplasm from the Triticeae Coordinated Agricultural Project will provide extremely diverse phene states and alleles for use for functional phenomics and genome wide association studies. The maize nested association mapping panel and soybean nested association mapping panel will be secured from current collaborator Felix Fritschi for future use. I will grow all these species in the field and phenotype for root architecture, root anatomy, shoot mass, shoot ion composition, and shoot architecture. The most contrasting phenotypes will be utilized in more detailed physiological studies in the greenhouse and in the field.

Intensive root crown phenotyping of grasses and legumes will be achieved using a custom root crown phenotyping pipeline that will substantially increase both the extent and intensity of phenotyping currently possible. Excavating, transportation, and washing of root crowns are major limitations that not have been addressed. Manual excavation in soft soils is relatively fast, however in harder soils use of a mini-excavator may be necessary. Root crowns collected in the field need to be transported with electric wheelbarrows or wagons. Washing roots can also be very laborious, but has the potential to be automated. I propose to build system that uses metal wire baskets to hold roots and automatically puts them through a soaking phase to soften soil, then moves inside a chamber with many water nozzles surrounding the roots to remove soil. The water used will be filtered and recycled as much as possible, whereas most washing stations simply lose water into a soggy field. Custom root imaging stations will be constructed in order to maximize user comfort, throughput, and the quality of data collected. Roots will be suspended in the center of a cylinder with the background walls coated in light absorbing black flocking paper used in telescopes. A high-speed video camera will quickly rotate around the roots to capture several frames of the roots in 360 degrees. In some cases, this may allow 3D tomography of reconstructed root systems, but more importantly this eliminates variation caused by user placement of roots that are not symmetrical. Eliminating this variation will increase the statistical power to resolve genotypic differences and the probability of finding QTL and gene candidates. Capturing high quality images from the field in a high-throughput manner will be a major breakthrough for the root phenotyping community, however using automated image analysis will be necessary to handle the volume of images. In order to capture the plot and genotype information in samples, I propose to use cheaply available RFID chips that can be read at a distance when then images are acquired, the file name will be saved with this information automatically.

Image analysis requires segmentation of the root system from the background, which necessitates high quality images where root pixels are substantially different from background. Image analysis software already exists from my previous work, but would require more work to integrate into this phenotyping pipeline efficiently. This software will automatically capture many properties that capture the extent and intensity of foraging, but future work is needed to dissect these properties into more elemental phenes such as the number of axial roots

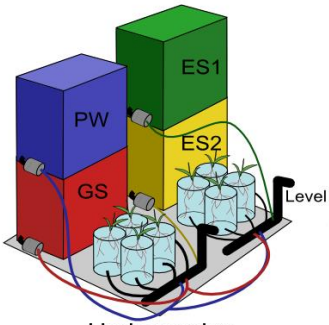
and lateral root branching density from particular classes. I will provide plans for the imaging stations and this software freely to the research community.

Anatomical phenotyping of roots offers the potential to link cortical phenes such as aerenchyma and number of cortical cells to plant metabolism, and stele phenes such as xylem number and diameter to resource uptake properties. **Laser ablation tomography** (LAT) is an exciting platform that uses a pulsed laser to ablate the root surface sequentially while the cross section is imaged between pulses. I would use my startup package to build an advanced LAT system that not only images anatomical structures, but determines their composition using hyperspectral imaging. Samples would be collected after the imaging steps described above from three locations in each root system, and several anatomical cross-sections imaged per root, again to reduce the variation of error terms in statistical models. RFID chips would travel with the samples in test tubes and be automatically scanned when the samples are imaged. Similar to the image analysis problems described above, analysis of anatomy pictures requires segmentation of cell walls from non-cell walls (the background). Only then can outlines be traced and anatomical features measured. Software exists that automates most steps, but future work will integrate similar software with my overall pipeline.


Less aerenchyma More aerenchyma

Phenotyping root system architecture and anatomy in the field will be my initial goal. More intensive measurements would be planned for subsets of the populations in the field, greenhouse, and laboratory. For example, cheaper root systems with fewer roots or less cortical burden from anatomical phenes should respire less in the field, which I propose to test directly by use of commonly available soil respiration chambers. Based on field data, I will soil core to 1.5 meters depth using a Giddings tractor-mounted soil corer the most contrasting root phenotypes in order to demonstrate a relationship between the root crown phenotypes and root length density distribution. From these plots, soil would be collected for water and elemental analysis to potentially link root length with soil resource depletion. Shoot samples would be collected from all field plots for mass determination and ion profiling to link root form to function.

I was recently awarded an EAGER grant for developing **high-throughput phenotyping of multiple ion uptake kinetics**, which could potentially transform root phenomics with an entirely new set of phenes. My previous work demonstrated substantial knowledge gaps in our understanding of how the number and activity of nutrient transporters integrate to give rise to total plant uptake rates. To address these gaps, simulation modelling of this detailed process is under development including an external solution, transporters at the root epidermis, and assimilation processes. My future work will leverage this new phenotyping platform in both maize and Arabidopsis to substantially increase our basic understanding of nutrient uptake and how to apply this information to breeding programs. This is an opportunity to start with very basic research suitable for NSF funding but that could quickly lead to advances in our knowledge that could be applied in the field.



Hydroponics


Peristaltic pump


Fraction collector

Ion chromatragraphy

My EAGER proposal aims to grow plants in hydroponics solutions across a range of nutrient concentrations and automatically sample that solution using Rasberry Pi computers linked to hobbyist peristaltic pumps. These samples will be analyzed using ion chromatography in order to calculate the depletion of nutrients. These data will allow the calculation of Michaelis-Menten parameters using curve fitting algorithms I have written in R. First, I will phenotyping the parents of the maize NAM panel, then then entire population from the two most contrasting parents. This strategy allows a smarter way to target phenotyping to more relevant lines. I will also measure root length, anatomy, pH, and respiration to generate a more complete understanding of uptake kinetics. If this phenotyping is combined with proteomic and transcriptomic analysis, we can make great progress in how nitrate transporters, ATPases, and the assimilation process influence nitrate uptake.

Simulation modeling using *SimRoot* will provide another important pillar to my research. For example, that fewer nodal roots contributes to greater nitrate efficiency in maize was first documented in my dissertation simulations and later confirmed in the field. Using simulations, we can grow plants *in silico* in far greater numbers of environments and phenotypes so that we can then focus our efforts on the phenes demonstrated to be most important in the model. At the same time, our empirical work updates the functioning of the model and the range of phenotypes being simulated. The computational model currently being developed for how transporter abundance and type influences uptake kinetics can be incorporated into *SimRoot* to create a more physiologically mechanistic uptake module.

Phenotyping large diversity panels will allow genome wide association studies (GWAS), however only identifying alleles with large effects obscures the underlying influence of allelic variation on the continuous natural variation we see in root properties. The 3000 rice genomes project with nearly 20 million SNPS offers one inspiration in crop science to think big and a similar project in one of our focal crops could substantially increase our ability to link gene to phene, and more importantly, link phene to functional utility. GWAS conducted on a diversity panel would allow the identification of genetic loci related to architectural, anatomical, and kinetics phenes. Collaborations with molecular biologists would allow identification of homologs in Arabidopsis and reverse genetics to manipulate gene expression and confirm GWAS results. Such mutants can also be used to confirm the functional utility of phenes, which is an underutilized research approach.

Through implementation of the root phenomics pipeline in forage and field crops including tall fescue, alfalfa, wheat, maize, and soybean substantial advances in root biology can be made relatively quickly. Though hypotheses have been made about root ideotypes, such as the ‘steep, deep, and cheap’ ideotype for maize efficient for nitrate uptake, little progress has been made in testing these hypotheses. The root phenomics platform combines hypothesis testing with hypothesis generation in a synergistic fashion that will maximize research output. **By harnessing creativity and technical prowess, we can use roots to improve soil and food security in Oklahoma and around the world.**