INTEGRATION OF ROOT PHENES AFFECTING NITROGEN ACQUISITION IN

MAIZE (ZEA MAYS)

A Dissertation in
Ecology
by
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ABSTRACT

Food insecurity is among the greatest challenges humanity will face in the 21st century. Agricultural production in much of the world is constrained by the natural infertility of soil which restrains crops from reaching their yield potential. In developed nations, fertilizer inputs pollute air and water and contribute to climate change and environmental degradation. In poor nations low soil fertility is a primary constraint to food security and economic development. Increasing the acquisition efficiency of soil resources is one method by which crop yields could be increased without the use of more fertilizers. Maize is one of the most widely grown crops, both in terms of land area and in yield, so optimizing uptake efficiency of maize is an important goal.

Roots are the primary interface between plant and soil and are responsible for the uptake of soil resources. The deployment of roots in space and time comprises root system architecture (RSA). Maize RSA is a complex phenotype that aggregates many elemental phenes (elemental units of phenotype). Integration of root phenes will be determined by interactions through their effects on soil foraging and plant metabolism. Many architectural, metabolic, and physiological root phenes have been identified in maize, including: nodal root number, nodal root growth angle, lateral root density, lateral root length, aerenchyma, cortical cell size and number, and nitrate uptake kinetics.

The maize root system is composed of an embryonic root system and nodal roots that emerge in successive whorls as the plant develops. Current phenotyping platforms often ignore the inner whorls and instead focus on the most visible outer whorls after excavating a maize root crown from soil. This dissertation researches the RSA among whorls of the maize nodal root system and demonstrates how the variation with the root crown could have functional significance. Nodal root number was decomposed to more elemental phenes including the number of nodes and the occupancies of each node. Simulations demonstrated that root systems forming fewer nodal roots and with delayed emergence perform well in low nitrogen soils. Nitrate uptake kinetics (NUK) vary within the maize root system, and simulations showing a lack of interaction between NUK and RSA reflects a knowledge gap in the costs of NUK at the molecular level. Finally, maize RSA among hybrids from different era periods over the past 100 years suggests evolution towards more nitrogen efficient states.
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Larry Matthew York
Chapter 1

General Introduction

Global food production must double in order to meet the demands of the future population of more than 9 billion people predicted by 2050 (Royal Society, 2009). New arable land is not plentiful (Pretty, 2008), so land use efficiency must increase dramatically to meet current and future demand. Increasing crop nutrient and water use efficiencies is one approach for increasing land productivity (Lynch, 1998). Maize (Zea mays L.) is one of the most important crops both in terms of its dominance in global agricultural land area and global yield (FAO, 2012) so increasing maize nutrient and water use efficiency is a desirable breeding goal. Nitrogen is a soil resource that is often limited to plant growth, that is costly to add as fertilizer, and that often pollutes the air and water (Hirel et al., 2007). The root system is the plant organ in direct contact with the soil and is responsible for the uptake of soil resources so research on root system properties that increase nitrogen use efficiency deserves attention (Lynch, 1995).

The maize root system is comprised of an embryonic root system consisting of the primary root and seminal roots that emerge from the seed, and successive whorls of nodal roots that emerge from the shoot (Hochholdinger, 2009). In the root system architectural taxonomy adopted by the International Society for Root Research, the primary root, seminal roots, and nodal roots of maize would be classified as the tap root, basal roots, and shoot-borne roots, respectively (Zobel and Waisel, 2010). Maize performance is influenced by many root system architectural, anatomical, and morphological phenes (i.e., elemental units of phenotype) and phene aggregates (see Lynch and Brown, 2012; and discussion in York et al., 2013) that influence root distribution and soil resource acquisition, including crown root number (York et al., 2013; Saengwilai et al., 2014b) topsoil foraging (Zhu et al., 2005a), crown root angle (Trachsel et al., 2013), rooting depth (Hund et al., 2008), lateral branching (Zhu and Lynch, 2004; Zhu et al., 2005b; Postma et al., 2014), root cortical aerenchyma (RCA) (Zhu et al., 2010a; Saengwilai et al., 2014a), living cortical area (Jaramillo et al., 2013), cortical cell size (Chimungu et al., 2014a), cortical cell file number (Chimungu et al., 2014b), and root hairs (Zhu et al., 2010b). Root phenes may also influence interplant competition and facilitation (Hodge et al., 1999; Ge et al., 2000; Bates and Lynch, 2001; Rubio et al., 2003; Postma and Lynch, 2012; Zhang et al., 2014). Interactions among root phenes
for foraging utility and metabolism economics will determine how these root phenes influence soil source acquisition together to create a functionally integrated phenotype (York et al., 2013). An ideotype of maize root phenotypes for water and nitrogen acquisition has been proposed consisting of several interacting architectural, anatomical, and physiological phenes (i.e., steep, deep, and cheap, or ‘SCD’; Lynch 2013).

This dissertation focuses on root architectural phenes affecting nitrate acquisition, and also the physiological phenes affecting nitrate uptake kinetics. Phenes that influence soil foraging exist on a spectrum from those that affect soil exploration to those that affect soil exploitation, with some phenes that are intermediate. Soil exploration by roots is characterized by growing into new soil domains beyond soil domains already depleted of resources, which corresponds to increasing the root system’s zone of influence (Casper et al., 2003). Exploration phenes include those that influence which soil domains are explored. Exploitation of soil resources describes how thoroughly resources are acquired within a given soil domain, i.e. with no further soil exploration. Exploration and exploitation are dependent on spatiotemporal scales and the mobility of the resource in question. Fitter proposed a measurement of acquisition efficiency to be the quotient of soil volume depleted to total root system volume (Fitter et al., 1991). This volume depends on the mobility of nutrients. Phosphorus depletion zones are only a few millimeters in diameter because phosphorus is relatively immobile in soil, while those for nitrate may be 10-100 times larger because nitrate is relatively mobile in soil due to the 1000 fold difference in effective diffusion coefficients (Barber, 1984). A phene state can increase exploration for an immobile resource by entering new soil domains, while also increasing the exploitation of a domain for a more mobile resource by increasing the intensity (inverse of efficiency) of its uptake. The differences in mobility between mobile and immobile nutrients give rise to root system depletion zone and root surface depletion zones, respectively (Bray, 1954). The growth angle of nodal roots influences the relative exploration of shallow and deep soil domains. Topsoil foraging is important for phosphorus acquisition in both maize and common bean (Lynch and Brown 2008), while deep soil foraging has been proposed to be important for the acquisition of water and nitrate (Lynch, 2013). When applied as fertilizer, especially, nitrate is a shallow resource in the early season, but to become deeper as it leaches during the growth season. Exploitation phenes affect the rate of nutrient uptake by increasing root density, rhizosphere modification, and increasing uptake kinetics. Rhizosphere modification includes decreasing the pH by releasing protons, organic acids, and by exudation of enzymes that release phosphorus from organic compounds (Lambers et al., 2006). Mycorrhizal symbioses can affect both exploration and exploitation, depending on the spatial scale and resource.
Mycorrhizal fungi increase soil exploration by the growth of their hyphae, and exchange phosphorus for carbon with their host plant (Harley, 1989). For the context of this dissertation, we generally expect architectural phenes that increase exploration over exploitation to be more important for nitrate uptake. However, uptake kinetics that increase the uptake of nitrate per unit root length with minimal cost will lead to increased soil exploitation for nitrate.

Root phenes within the mature root crown of maize are difficult to study because the outer whorls of the maize root system are the youngest roots and occlude the older roots in the interior. When root phene differences among nodes are studied only one line may be reported (e.g. Picard et al. 1985), or else an incomplete measurement of only a few nodes is conducted for many lines (e.g Guingo et al. 1998). One approach to increase throughput of field phenotyping is the use of digital images of excavated root crowns combined with automatic image analysis (Grift et al., 2011; Bucksch et al., 2014), however the use of mature root systems prevents measurements of the occluded part of the root system, and neither of these image analysis platforms count individual nodal roots, which contributes to the important phene aggregate of nodal root number (NRN). However, imaging of mature root crowns from the field can be conducted while excising whorls of nodal roots to reveal interior whorls, and nodal roots can be counted manually and angles measured using software. Imaging in the field saves substantial time, and while software-aided manual measurements take more time than completely automated image analysis, more precise measurements can be made in some sense because the human eye is currently better at detecting different classes of roots, especially with complex three-dimensional systems where roots occlude other roots.

Objectives

The objectives of this dissertation regarding the integration of root phenes affecting nitrogen acquisition in maize are as follows:

a) Clarify the language regarding the study of the root system phenotype and functional utility and develop a theoretical framework for understand phene integration (Chapter 2).

b) Identify variation within the root system for nitrate uptake kinetics and study the integration of root architectural phenes and nitrate uptake kinetics using simulation modeling (Chapter 3).
c) Extend the shovelomics phenotyping platform to accommodate intensive phenotyping of the maize root crown for several architectural phenes in every node of shoot-borne roots and establish the importance of integration of root phenes in the field (Chapter 4).

d) Study the interaction of nodal root number and nodal root growth angle in more detail using simulation modeling (Chapter 5).

e) Test the hypothesis that maize root system architecture has evolved over the past 100 years towards more nitrogen efficient phene states because of indirect selection through breeding programs in changing agronomic conditions (Chapter 6).

References


Chapter 2

Integration of root phenes for soil resource acquisition

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Abstract

Suboptimal availability of water and nutrients is a primary limitation to plant growth in terrestrial ecosystems. The acquisition of soil resources by plant roots is therefore an important component of plant fitness and agricultural productivity. Plant root systems comprise a set of phenes, or traits, that interact. Phenes are the units of the plant phenotype, and phene states represent the variation in form and function a particular phene may take. Root phenes can be classified as affecting resource acquisition or utilization, influencing acquisition through exploration or exploitation, and in being metabolically influential or neutral. These classifications determine how one phene will interact with another phene, whether through foraging mechanisms or metabolic economics. Phenes that influence one another through foraging mechanisms are likely to operate within a phene module, a group of interacting phenes, that may be co-selected. Examples of root phene interactions discussed are: 1) root hair length × root hair density, 2) lateral branching × root cortical aerenchyma, 3) adventitious root number × adventitious root respiration and basal root growth angle, 4) nodal root number × root cortical aerenchyma, and 5) basal root growth angle × root hair length and density. Progress in the study of phenes and phene interactions will be facilitated by employing simulation modeling and near-isophenic lines that allow the study of specific phenes and phene combinations within a common phenotypic background. Developing a robust understanding of the phenome at the organismal level will require new lines of inquiry into
how phenotypic integration influences plant function in diverse environments. A better understanding of how root phenes interact to affect soil resource acquisition will be an important tool in the breeding of crops with superior stress tolerance and reduced dependence on intensive use of inputs.

Introduction

Global food security is a serious challenge (Funk and Brown, 2009), with approximately 870 million people experiencing chronic undernourishment (FAO et al., 2012). In much of the developing world, use of nitrogen (N) and phosphorus (P) fertilizers is relatively low, leading to substantial reductions in crop yields (FAO, 2008). In developed nations intensive use of fertilizers is associated with greater crop yields (Roberts, 2009). However, crop plants in these agricultural systems take up only a portion of the applied nitrogen fertilizer (Goulding, 2000), and the remainder pollutes water and the atmosphere (Jenkinson, 2001). Furthermore, phosphorus fertilizers are a non-renewable resource, and global production of phosphorus is expected to peak around the year 2033 (Cordell et al., 2009). Increasing crop acquisition of both nitrogen and phosphorus is therefore a desirable goal for both subsistence and commercial agriculture. At the same time, belowground properties of natural ecosystems are also demanding attention because of their influence on carbon sequestration (Eissenstat et al., 2000) and community structure (Craine et al., 2002).

Root architecture, the spatial arrangement of a root system, has been shown to be important in agricultural systems (Lynch, 1995; Ho, 2004; Hirel et al., 2007) and natural systems (Mahall and Callaway, 1992; Comas and Eissenstat, 2009) for nutrient acquisition, plant interactions, and nutrient cycling. Understanding the contribution of specific root traits, or phenes, to root system function is critical for crop improvement because it allows identification of traits that contribute desired functions (Kell, 2011; Lynch and Brown, 2012). High-throughput root phenotyping is an important tool in this context as it permits the profiling of the extent, magnitude, and distribution of root traits in crop germplasm, and because phenotyping is limiting progress in crop breeding (Furbank and Tester, 2011). Advances in high-throughput phenotyping of roots (Trachsel et al., 2011; Grift et al., 2011; Zhu et al., 2011) will enable focused efforts to improve crop nutrient acquisition by selection for root ideotypes and to understand the influence of inter-and-intraspecific root system variation on community structure and ecosystem function.
Ideotype, or trait-based, breeding was proposed by Colin M. Donald as a way to combine traits that would each contribute to increased yield (Donald, 1968). He identified a flaw in “deficit elimination” or “selection for yield” approaches in that they do not seek to answer how increased yield is created (Donald, 1962). Instead, he proposed studying traits in isolation to understand how they contribute to yield then combining such yield improving traits through traditional breeding. Crop breeding programs commonly combine traits, especially in the pyramiding of traits associated with disease resistance (Shen et al., 2001; Singh et al., 2001; Steele et al., 2006). This approach has contributed substantially to yield gains in several crops, including maize, wheat, and common bean (Mock and Pearce, 1975; Kelly and Adams, 1987; Reynolds et al., 1994; McClean et al., 2011). The trait-based approach inherent in the concept of ideotype breeding forced researchers to not only consider traits of interest in isolation, but also to consider relationships among traits. This is illustrated by the work of Rasmusson (1987), demonstrating that compensation among plant organs can lead to tradeoffs, such as increasing head numbers being associated with fewer, smaller kernels in barley. The integration of traits determines how the whole plant functions and remains an underutilized aspect of ideotype breeding.

A body of work on phenotypic integration in the field of evolutionary biology and ecology has also considered some aspects of the relationships among traits (Murren, 2002; Pigliucci, 2003). In this context phenotypic integration has been defined as the ‘pattern of functional, developmental and/or genetic correlation (however measured) among different traits in a given organism (Pigliucci, 2003). In plants, this area of research originated with the work of Berg (1960) who identified clusters of correlated traits. Strong correlations between traits could imply shared functions, with correlations among traits possibly maintained by stabilizing selection. In some cases researchers have focused on how groups of correlated traits affect plant function in specific ecological contexts (Lechowicz and Pierre, 1988). Economic spectrums that relate traits by their costs and functions have been identified in leaves (Wright et al., 2004), and proposed for roots, though evidence for a root economic spectrum remains inconclusive (Chen et al., 2013). In this research phenotypic diversity within species or populations has typically been viewed as noise rather than as an important response to heterogeneous and unpredictable environments, competition, and phenotypic plasticity. Both ecological and agricultural research have converged upon concepts of integration through genetic, physiological and developmental correlation (Grafius, 1978), though researchers in both areas seem to be largely unaware of the other.

Trait ‘stacking’ in genetically modified crops (GMCs) is another form of ideotype breeding and trait integration. Traits of interest here are usually of the “deficit elimination” type, such as
reducing susceptibility to insects or herbicides. First-generation stacks included \textit{Bt} toxin-producing and glyphosate-resistant GMCs that were introduced in 1998 (James, 2000). In order to decrease the selection for \textit{Bt} toxin resistance in agricultural insect pests, 2\textsuperscript{nd}-generation stacks combine several modes of actions for the same trait, which also reduces requirements for non-GMC refuge areas (Que et al., 2010). Stacking technologies have rapidly developed to higher numbers of combined traits, such as the 9 foreign proteins combined in \textit{SmartStax™} (Marra et al., 2010). Gene stacking does lead to trait interactions in that most GM traits enhance growth in some situations, and combining modes of action decreases the ability of pests to adapt. Trait synergisms have been considered by biotechnology companies (Then, 2011), but only in terms of multiple modes of action for pest control, similar to the pyramiding of genes for disease resistance through introgression breeding.

Traditional plant breeding has attempted to combine traits that are helpful in isolation, and transgenic crops have also made progress in the stacking of particular traits. Ecologists have observed correlations among traits and between traits and plant function. However, our understanding of non-additive trait interactions is limited, and this is particularly true in root biology. Here we propose a theoretical framework for evaluating root system phenes and their functional interactions in the context of soil resource acquisition. We will show that the combining of traits does not always lead to a simple accumulation of additive effects, so plant biologists and breeders must take into account trait synergisms.

\textbf{Theoretical Framework}

\textbf{What is a phene?}

‘Phene’ was used as early as 1925 in animal genetics to describe phenotypic traits under genetic control (Serebrovsky, 1925), and has been used extensively in European and Russian agricultural literature (e.g., Gustafsson et al., 1977). Phene can be defined concisely: \textit{phene} is to \textit{phenotype} as \textit{gene} is to \textit{genotype} (Lynch, 2011; Pieruschka and Poorter, 2012). Just as genes have variants called alleles, phenes have variants we will refer to as \textit{phene states} (\textit{phene} is to \textit{trait} as \textit{phene state} is to \textit{attribute}). The particular combination of states for all phenes constitutes the phenotype of an individual organism. We will use \textit{phenome} as the totality of all possible phene
states of a taxon, i.e., phenotypic potential (Figure 1). Alternative more generic terms such as traits, characters, and attributes have been used with ambiguity that can lead to confusion (Violle et al., 2007), such as by referring to properties at several levels of biological organization or by using trait to refer to either phenes or phene states. Lynch and Brown (2012) proposed that the most useful and meaningful phenes are elementary and unique at their level of biological organization (e.g., organ, tissue, cell). For example, an elementary root architectural phene should not be the product or aggregation of other more basic architectural phenes. The genetic and developmental processes giving rise to phenes should be unique, i.e., a phene is elemental because it has a unique developmental pathway. Some phenes may be under single gene control, and have phene states that are discrete. Many phenes are probably quantitative traits resulting from the interaction of many genes and the environment, and will show a continuous distribution of phene states. Many measurements of plant phenotypes are aggregates of multiple elemental phenes. For example, rooting depth has been shown to be influenced by separate phenes, such as root growth angle (Trachsel et al., 2013) and aerenchyma (Zhu et al., 2010a). Such plant characteristics may be referred to as phene aggregates. Plant measurements similar to yield, plant mass, or nutrient content will not be referred to as phenes or as phene aggregates. Rather, they are functional responses dependent on the state of many components of the plant phenotype.

Phene states make up phenotypes, which are individual manifestations of the phenome of a species. The root phenes of interest to us here have functional utility for resource acquisition (Lynch 2011), and are components of root architecture, morphology, anatomy, or physiology. In turn, these functions influence agricultural performance such as biomass and yield, or plant fitness in natural systems (Figure 2), sensu Arnold (1983) and Violle et al. (2007). Functional utility can be assessed by comparing the functional responses of different phene states. For example, it has been shown that plants with longer root hairs acquire more phosphorus than plants with shorter root hairs or none at all (Bates and Lynch, 2000; Yan et al., 2004; Zhu et al., 2010b). The comparison of the phosphorus acquisition responses of these two root hair phene states demonstrates that the root hair length phene is important for P acquisition, with longer root hairs leading to greater P acquisition. A phene-function response curve shows the influence of a single continually varying phene on a plant function (Figure 3).
Root phene classification

**Root phenes classified by function, foraging strategy, and metabolic influence**

Phenes can be classified in numerous ways. A mechanistic classification of root phenes can be made on the basis of whether they affect resource *acquisition* or resource *utilization*. Phenes that affect soil resource acquisition generally affect the coincidence of root foraging and soil resource availability in time and space. Phenes that affect resource utilization influence how efficiently resources are used for plant functions including growth, further resource acquisition, and reproduction. Phenes that affect resource acquisition can be further classified based on foraging strategy. Foraging strategies exist along a continuum from phenes that influence soil exploration to those that influence soil exploitation. Exploration phenes influence the spatial and temporal exploration of soil domains by roots and root symbionts. Exploitation of soil resources describes how thoroughly resources are acquired within a given soil domain, i.e., with no further soil exploration. Fitter proposed a measurement of acquisition efficiency to be the quotient of soil volume depleted to total root system volume (Fitter et al., 1991). This volume depends on the mobility of nutrients. Phosphorus depletion zones are only a few millimeters in diameter, while those for nitrate may be 10-100 times larger due to the 1000 fold difference between phosphate and nitrate in effective diffusion coefficients (Barber, 1984). A phene state can increase exploration for an immobile resource by entering new soil domains, while also increasing the exploitation of a domain for a more mobile resource by increasing the intensity of its acquisition (Figure 4). The differences in mobility between mobile and immobile nutrients give rise to the *root system depletion zone* and *root surface depletion zones* (lighter grey versus dark grey in Figure 4), respectively (Bray, 1954). The growth angle of axial roots (e.g. nodal roots in maize, basal roots in common bean) influences the relative exploration of shallow and deep soil domains. Topsoil foraging has been shown to be important for phosphorus acquisition in both maize and common bean (Lynch and Brown 2008), while deep soil foraging has been proposed to be important for the acquisition of water and nitrate (Lynch, In Press). Exploitation phenes affect the rate of nutrient uptake by increasing root density (number or length of roots in a volume) through greater numbers of axial roots, lateral branching, or root hairs and rhizosphere modification, for example. Rhizosphere modification includes decreasing the pH by releasing protons, organic acids, and by exudation of enzymes that release phosphorus from organic compounds (Lambers et al., 2006).
Mycorrhizal symbioses can affect both exploration and exploitation, depending on the spatial scale and resource. Mycorrhizal fungi increase soil exploration by the growth of their hyphae, and exchange phosphorus for carbon with their host plant (Harley, 1989). Resource acquisition phenes not only differ in foraging strategies but in how they influence plant metabolism, and effects on metabolism are the mechanism for utilization phenes.

The functional utility of root phenes for soil resource acquisition is strongly influenced by rhizoeconomics (Lynch and Ho 2005; Lynch 2007a), i.e., their relative costs and benefits. One of the major costs of roots is their metabolic demand. Several economic currencies can be used to estimate cost/benefit relationships, such as carbon, nitrogen and phosphorus (Lynch and Rodriguez H., 1994; Lynch and Beebe, 1995). Metabolic costs can be partitioned into construction and maintenance costs (Chapin III et al., 1987). Root construction costs are generally strongly influenced by root volume which is proportional to length and diameter, so phenes which determine these (e.g. elongation rate, branching, number of roots formed, and root diameters) will influence construction costs. Roots, like all plant tissues, require not only carbon, but also mineral nutrients for construction and maintenance. Phenes have been identified that alter root metabolic demand. ‘Root etiolation,’ or decreasing diameter in order to increase length, has been proposed as an adaptive trait for nutrient acquisition (Lynch and Brown, 2008), with empirical support provided in maize (Zhu and Lynch, 2004). Root cortical aerenchyma converts living cortical tissue to air space via programmed cell death. This lowers the respiration of root segments (Fan et al., 2003), and has the additional benefit of mobilizing nutrients for other uses (Postma and Lynch, 2010). An economic classification of root phenes is based on how they influence metabolism. Table 1 presents a number of root phenes and their classification according to these three schemes (acquisition vs. utilization, exploration vs. exploitation, and metabolic influence vs. no metabolic influence).

**Not all root measurements are root phenes**

A multitude of root measurements are made in both agricultural and natural systems that do not meet the definition of an elemental phene. Rather, most of these root measurements represent phene aggregates that are influenced by the states of several root phenes (Table 2). Others, such as total root length, are *functional responses* that are influenced by states of phenes through their influence on soil resource acquisition and eventual photosynthate allocation to the root system. Unexplained variation in these measurements may be resolved by more thorough documentation.
of constituent root architectural, anatomical, and physiological phenes. These measurements may often be referred to as traits, which highlights the difference between the common usage of trait and the biological definition of phene.

**How do phenes interact?**

*Functional response interactions*

The utility of a phene can be assessed by comparing the functional responses of varying states of the phene. Similarly, the interaction of two phenes can be assessed by combining at least two phene states of two different phenes and measuring the functional response of each combination. In such a situation, the null hypothesis is that the functional response of two phene states from two different phenes will be additive. The particular phene state combination is synergetic when the functional response exceeds the sum of the responses of the phene states in isolation. Antagonistic interactions occur when the functional response of phene states in combination is worse than that expected from the sum of their responses in isolation. We can describe the mechanistic basis of the interaction based on the classifications of the component phenes. A phene-function response landscape graphically demonstrates how the simultaneous changes of two or more phenes affect a function (Figure 5).

*Foraging strategy interactions*

Phenes interact through their effects on foraging when the mechanism through which one phene affects foraging directly interacts with the mechanism of another phene affecting foraging. For example, axial roots with shallow growth angles will increase the exploration of soil with greater amounts of phosphorus, while increased root hairs will increase the exploitation of the explored soil. The combined states of shallow growth angles and increased root hairs may have a synergetic interaction beyond what would be expected based on their additive effects on phosphorus acquisition (see Case Study 2).
Economic interactions

The economic interaction of two phenes is mediated by the metabolic budget of the plant. Two metabolism influencing phenes will exhibit tradeoffs when occupying more metabolically demanding states. These tradeoffs are expected between root classes, or even between number and length within a class (Walk et al., 2006; Rubio and Lynch, 2007). Building more of one type of root will necessarily limit the metabolic resources available for building other types, or decrease the resources available for elongation of existing roots. However, feedbacks between nutrient acquisition and increased photosynthesis that allow further root growth are possible. Conversely, a metabolically neutral phene will have no economic interaction with a metabolism influencing phene.

Phene modules

Combinations of specific phene states may be more likely to be found together in individuals of a taxon when they act as a functional module through foraging and economic interactions. Modules are aggregates of components that are related, such as in the context of molecular pathways (Hartwell et al., 1999), architectural modules such as leaves, flowers, and roots, and even entire plants as modules in an ecosystem (Prusinkiewicz, 2004). One useful definition for module in the context of phene interactions is a group of phenes that behave synergistically. In roots, such functional module components probably belong to the same parent root class, similar to the ‘modular unit’ suggested by Pregitzer et al. (2002) as lateral branches of tree roots consisting of several orders of the finest roots. In crops such as common bean and maize, these modules are initiated from and include the major axes, i.e., basal roots in bean, nodal roots in maize. Foraging interactions are more likely to occur in modules composed of phenes that are close together because their likelihood of coinciding with a soil resource increases.

Environmental interactions

It is well known that the abiotic and biotic environments can affect the phene states of an organism through the phenomenon of phenotypic plasticity (West-Eberhard, 1989; Callaway et al.,
2003). For example, roots have been observed to proliferate in patches of nutrients (Drew and Saker, 1975; Granato and Raper, 1989), change rooting angle (Bonser et al., 1996), change root hair density (Ma et al., 2001a), and alter axial elongation and lateral root density in response to phosphorus availability (Borch et al., 1999). Root phenotypic plasticity constitutes one type of phene-environment interaction. Another type is based on tradeoffs and synergies that may exist between root phenes and particular soil resources, i.e., phene × environment × functional response interactions. For example, in low phosphorus soils, phenotypes with shallow root growth angles perform better than phenotypes with steep root growth angles, but in high phosphorus conditions both perform equally well. Steep-angled phenotypes are better at acquiring water during terminal drought (Ho et al., 2005), so there is an architectural tradeoff for root growth angle for acquiring resources at different depths in the soil. When both phosphorus stress and terminal drought occur together, shallow-rooted phenotypes performed better because early P uptake allowed the growth of more extensive root systems that then conferred greater tolerance to the terminal drought. Phene × phene × environment interactions are more complicated than single phene × environment interactions, but must be studied in order to understand how plants cope with multiple stresses, and how suites of traits influence fitness.

**Interplant interactions**

Root competition among plants of different species plays an important role in shaping plant communities (Schenk, 2006) and in the performance of interspecific polycultures in agriculture (Wilson, 1988; Postma and Lynch, 2012). Competition is expected to be greater for mobile nutrients than relatively immobile nutrients (Postma and Lynch, 2012; Wilberts et al., In Press). Little is known about how specific root traits affect competition and facilitation, but there are a few examples. *Arabidopsis* wildtypes with root hairs were shown to have a competitive advantage over root hairless *rhd2* mutants in low phosphorus media (Bates and Lynch, 2001). Similarly, *Arabidopsis* wildtypes out-competed *axr4* mutants with decreased numbers of lateral roots in low phosphorus, but not in low nitrogen (Fitter et al., 2002). Architectural multilines of common bean composed of equal portions of plants with shallow and steep basal root angles had Land Equivalent Ratios greater than unity (Henry et al., 2010), which means more area must be planted of the monocultures in order to achieve the same levels of yield as the multilines. This implies a competitive release of the dominant shallow-rooted plants when grown with steep-rooted plants in
low phosphorus soils. Common beans were shown to alter root architecture in the presence of neighboring plants due to localized phosphorus depletion (Nord et al., 2011). Clearly, understanding phenes requires an understanding of how phenes will react to other phenes, the environment, and other plants.

**Phene Integration**

Foraging, economic, environmental, and interplant interactions of phenes create an integrated phenotype. The integrated phenotype is more than simply a collection of isolated traits, but rather is a suite of interacting phenes that affect plant functions. These interactions cannot simply be assumed to be additive and will depend on the environmental context. Phene integration occurs at all levels of phenotypic organization, from cells, to modules, to the whole plant.

Phenes may interact via resource partitioning and signaling, even between roots and shoots. Typically, shoots provide photosynthates to the roots, while roots supply soil resources to the shoot. Thornley (1972) developed a mathematical model with two pools, shoot and roots, and two substrates, carbon and nitrogen, which are supplied by the shoot and roots, respectively. This simple source-sink model demonstrated that plants should balance shoot and root activity and invest in the organs that produce the most limiting resource, and continues to guide whole plant modeling. Empirical work demonstrates that aboveground and belowground organs communicate their internal and environmental status to each other in order to integrate plant function in dynamic environments. For example, root ABA signals induce stomatal closure in leaves which decreases transpiration (Davies and Zhang, 1991). The plant shoot is partially responsible for perceiving the internal nitrogen status and uses reduced nitrogen compounds and auxin to signal roots to form lateral roots (Ruffel et al., 2011). Interestingly, roots can also influence shoot branching through auxin signaling (Bennett et al., 2006), which might suggest root perception of the soil environment informs the regulation of shoot growth. These interactions suggest that another form of phene interaction may be information exchange, which may apply within the root system as well. The global leaf economic spectrum demonstrates that leaves from a variety of species representing diverse functional groups are constrained by development and natural selection to fall along a single spectrum for a variety of traits (Wright et al., 2004). A direct interaction between a shoot phene such as leaf morphology and an RSA phene like lateral branching is unlikely. Rather, the shoot and
root organs integrate information processing and metabolism, and balance production of photosynthates with acquisition of soil resources (Figure 6).

Hypotheses regarding the integration of root phenes

We propose the following hypotheses regarding the integration of root phenes:

1. Functional synergisms will occur among foraging phenes that act within a module including the axial root and its subordinate roots.

2. Functional synergism will increase as the number of positively acting phene states combined is increased.

3. Metabolic tradeoffs will limit synergism created by combining foraging phene states that demand more metabolic resources, except when alleviated by phenes in states that relieve metabolic constraints.

4. Synergisms will be more likely to occur when combining metabolically neutral phenes in positively acting states.

5. The large diversity of root system phenotypes, i.e., the particular combination of phene states of an individual, is partially explained by the interactions of phenes within plants, between plants, and between phenes and the environment.

Case Studies

Research on phene interactions is nascent, and this is especially true in the case of roots. Much of the evidence for root phene integration comes from research with SimRoot, a functional-structural plant model focusing on root system architecture and nutrient acquisition (Lynch et al., 1997; Postma and Lynch, 2010), though we will also discuss empirical evidence and experimental approaches for studying phene interactions.
Root hair length x Root hair density

Root hairs are subcellular extensions of root epidermal cells that are particularly important for the acquisition of immobile nutrients such as phosphorus. Root hairs can vary in density (i.e., number of root hairs per unit root surface area) and in length. Diversity for both of these traits is evident in several species including common bean, soybean, and maize (Wang et al. 2004; Yan et al. 2004; Zhu et al. 2005). SimRoot was employed to test interactions among root hair length, root hair density, proximity of root hair appearance to the apical meristem, and the spatial patterning of hair-bearing cells (trichoblasts) and non-hair-bearing cells (atrichoblasts) in *Arabidopsis* (Ma et al., 2001b). The synergetic effect of increased root hair length and density phene states was 272% greater than their expected additive effects. Root hair formation nearer the root tip increases P acquisition, while number of files had positive effects when more numerous. All positive phene states were compared to their expected additive function response in two-way, three-way, and four-way combinations. On average, synergetic effects increased with the number of positive interactions: two-way, 168%; three-way, 232%; and four-way, 371% greater than additive effects (new calculations from original data). Changing root hair length and density in *Arabidopsis* had no direct effect on root respiration (Bates and Lynch, 2000). We hypothesize that metabolically neutral phenes will have the greatest synergisms because of the lack of economic tradeoffs. As this example shows, the magnitude of phene synergisms may increase with the number of positively interacting phene states (Hypothesis 2).

Lateral branching x Root cortical aerenchyma

Variation for lateral root length and density has been observed in both the primary root and nodal roots of maize (Zhu et al., 2005b; Trachsel et al., 2011). Greater lateral root length and density would permit greater soil exploration, and so would improve acquisition of soil resources. However, increased lateral branching has high metabolic demand, and due to competing sinks it could influence the growth of other root classes. This trade-off could be alleviated by decreasing metabolic demand in other ways. SimRoot was used to test the hypotheses that increased lateral root branching would increase N and P acquisition and that this phene would be affected by the formation of aerenchyma (Postma and Lynch 2011). At the lowest level of nitrogen, there was a 42% reduction in shoot dry weight compared to the expected additive effects of increasing lateral
root branching and forming aerenchyma, which constitutes a functional antagonism. However, at
the intermediate level of nitrogen a synergetic interaction 220% greater than the expected additive
effects was observed. In the low phosphorus condition, the synergetic interaction was 33% greater
than the expected additive effects. This broad range of interaction demonstrates the importance of
environmental context.

Adventitious root number x Adventitious root respiration and Basal root growth angle

Adventitious roots emerge from the hypocotyl in common bean and have less construction
and maintenance costs than basal roots (Miller et al., 2003). Adventitious roots emerge in the
topsoil and typically have extremely shallow growth angles, so they were hypothesized to be an
adaptive trait for topsoil foraging. Basal roots are the principal axial roots in common bean
(Phaseolus vulgaris), and a shallow growth angle for basal roots has been shown to be important
for topsoil foraging (Bonser et al., 1996; Liao et al., 2004; Ho et al., 2005; Henry et al., 2010).
Adventitious roots were found to have a range of respiration rates from the same as tap roots, to
400% greater than tap roots (Bouma et al., 1997; Walk et al. 2006). Because phosphorus has low
soil mobility, it accumulates in the topsoil from the deposition of senesced plant tissue (Anderson,
1988). Both functional response and economic interactions were expected between adventitious
root number (ARN) and adventitious root respiration, and between ARN and basal root growth
angle (BRGA), which was tested in SimRoot (Walk et al., 2006). Increasing ARN greatly increased
phosphorus acquisition when adventitious root respiration (ARR) was the same as tap root
respiration, and marginally benefited phosphorus acquisition when ARR was two times tap root
respiration. When adventitious root respiration was four times greater than tap root respiration,
there was a negative relation between increasing ARN and phosphorus acquisition. At the highest
level of adventitious root respiration, not enough metabolic resources were available for the
construction of root length adequate for phosphorus acquisition. This shows a functional response
antagonism between greater states of ARN and ARR that is mediated through an economic
interaction. Adventitious root number was also expected to interact with basal root growth angle.
However, only additive effects were observed between greater ARN and more shallow basal root
growth angle, which suggests adventitious roots and basal roots function as independent modules
(Hypothesis 1).
Nodal root number x Root cortical aerenchyma

Unpublished results from SimRoot show interaction between root cortical aerenchyma (RCA) and number of nodal roots in maize (Figure 7). Across a range of N and P availability, root length and total biomass were strongly affected by nodal root number. RCA had little to no effect on biomass or root length when there were fewer than optimal crown roots, but increased root length and biomass with optimal or greater than optimal numbers of nodal roots, especially with suboptimal N or P. Because optimal nodal root number differed between N deficient and P deficient conditions, the range of nodal root numbers where RCA increased biomass depended on the environment. At medium levels of nitrogen and phosphorus, the synergetic effects of greater numbers of crown roots and RCA were 31.6% and 132% greater than the expected additive effects, respectively.

Basal root growth angle x Root hair length and density

In common bean, basal root growth angle (BRGA) is a soil exploration phene and was hypothesized to influence the utility of the root hair phene, which affects exploitation, by determining the placement of root hairs in the soil profile. A field study was conducted in Mozambique, comparing three recombinant inbred lines (RILs) for each of four phenotypes representing all combinations of shallow and deep BRGA and low and high root hair length and density (RHLD) (Miguel, 2012). In low P soil, shallow BRGA increased shoot growth by 57.7%, and greater RHLD increased shoot growth by 89.3% (Figure 8). Shoot mass of the combined positive states (shallow angle and greater RHLD) was 298% greater than the base line (steep angle and lower RHLD), which is twice the expected additive effect. Root hairs along with the basal roots or basal root laterals on which they form constitute a functional module which gives rise to high levels of synergism (Hypotheses 1 and 4).

Evidence for root phene function and interaction in natural domains

Variation in root phenes has been observed among wild species along with correlation between phenes, such as between specific root length and lateral branching (Comas and Eissenstat,
Differences in rooting depth among grassland species has been proposed as one contribution to the relationship between biodiversity and ecosystem productivity by allowing plants to exploit particular soil niches (Fargione and Tilman, 2005). As noted above, rooting depth is a phene aggregate influenced by rooting angle, number, and total metabolic allocation to the root system, so diversity for rooting depth among species influencing productivity represents phene x phene x species interactions. A suite of functional traits associated with acquiring nitrogen in nitrogen-limited grassland plants was proposed which included high carbon:nitrogen tissue, slow metabolic rates, and large root length (Craine et al. 2002). McCormack et al. (2012) found relationships across 12 tree species among root morphology, root chemistry, root lifespan, and whole plant traits, though in another study no clear relationship between root traits such as root diameter and nitrogen concentration was identified (Chen et al., 2013). These studies in natural systems demonstrate a growing awareness of the identification of a root economic spectrum that would be a useful tool for understanding variation in root systems. However, to our knowledge, examples are lacking demonstrating the interactions of specific root phenes for specific functions in natural systems. Most studies rely on interspecific diversity to create root phene variation, which confounds specific phenes with many other covarying factors. Below, we will discuss general approaches to study root phenes and root phene integration that can be extended to any study system.

**Gaps identified by comparing known interactions to possible interactions**

These case studies demonstrate progress in understanding root phene integration. Most of the studies have been conducted with simulation modeling so the work must be confirmed by empirical work but the work of Miguel et al. (2012) with basal root angle and root hairs is a notable exception where root phene state synergisms were demonstrated in agricultural fields. There are no examples of interactions where resource acquisition phenes affecting metabolic economy, such as axial root number and lateral branching, have been simultaneously manipulated, though Walk et al. (2006) showed an interaction between adventitious root number and respiration mediated through architectural tradeoffs with lateral roots of basal and tap roots. Foraging phenes that influence metabolism may have only additive, or even antagonistic, interactions because of tradeoffs in metabolic economy (Hypothesis 3). Further work is also needed to understand how phenes integrate within and between functional modules.
Approaches for Studying Phene Integration

Many studies analyzing plant traits have relied on comparisons between species for phene state variation and in natural environmental gradients for differences in abiotic conditions. However, such comparisons are confounded by the multitude of differences that exist among species and environments. The use of structured genetic populations that vary for specific phenes but share a common genetic background, evaluated in environments in which specific stresses are imposed, is a more powerful approach when possible (Lynch, 2011). This strategy has the advantage of allowing the comparison of different phene states within a common genetic and phenotypic background, which is especially important given our lack of understanding of phene integration. Populations of recombinant inbred lines (RILs) have been used both for genetic mapping and for near-isophenic comparisons in common bean and maize (Zhu et al., 2005a; Ochoa et al., 2006; Zhu et al., 2006; Yan et al., 2004; Zhu et al., 2005b; Ho et al., 2005; Henry et al., 2010). Near-isophenic lines refer to lines that differ primarily in the state of a single phene, or at least a small number of phenes. Populations of near-isophenic lines may also contain plants with combinations of phene states that allow the study of phene integration. Single gene mutants may not always be useful for studies of phenes because many phenes of interest are controlled by several QTL or genes (Lynch, 2011). While biparental RIL populations are useful for these phenotypic contrasts, their limited diversity (descending from two parents) may not allow the measurement of the breadth of the root phenome. Diversity panels representing broader variation in crops are now being used to probe the breadth of the root phenome. High-throughput phenotyping must increase in extent and intensity (Houle et al., 2010). Extensive phenotyping is accomplished through the sampling of larger numbers of plants of greater diversity. Intensive phenotyping is the measurement of more traits for each sample. Both are benefitting from the application of remote sensing, image analysis, and robotics (Fiorani and Schurr, 2013), including with roots (Galkovskyi et al., 2012). Intensity will be further increased by the inclusion of function-valued traits, or phenes that are best described as mathematic functions rather than single values (Kingsolver et al., 2001). Both extensive and intensive phenotyping will contribute to plant phenomics and the study of root phene integration.

Plant phenomics is generating vast amounts of data, and increases in the extent and intensity of phenotyping will accelerate the pace of data collection. The creation and use of data repositories by teams of scientists is imperative. In order for this data to be useful, it must include metadata (higher level information that describes the data and its context). Metadata has the benefits
of increasing data longevity and recycling by the creator and others (Michener, 2006). Metadata for functional-structural phenomics must include ontologies for identifying plant structures and research context (Illic et al., 2007; Madin et al., 2008). Root functional phenomics should include ontologies for roots that represent their phylogeny, genetics, and development (Zobel, 2011), but also their function. Root phenomics won’t mature without thorough documentation and sharing of data, especially due to the significant financial costs of root phenotyping.

Rasmusson (1987) proposed developing a ‘germplasm bank of ideotype traits’ where breeders would agree to cooperate to introgress phenes of interest into elite genetic backgrounds. Diversity in crop species traits is often found in landraces or other unimproved varieties (Bayuelo-Jimenez et al., 2011). Recently, Burton et al. (2013a; 2013b) reported substantial variation among RILs, maize landraces and teosintes for both root architectural and root anatomical phenes that could be of use in maize breeding. However, these unimproved genetic backgrounds act as barriers to the inclusion of phenes that comprise a desired ideotype for breeding programs. A collaborative network of plant physiologists and breeders working to identify and understand phenes useful for crop performance would benefit from germplasm banks containing phene states in common genetic backgrounds. In order for researchers and breeders to be able to choose appropriate material for their programs, integration of phenomic and germplasm bank databases will be required. Greater collections of such plant material and relevant genetic resources are available for crop species than for wild plants, but model systems such as *Arabidopsis* and *Populus* may act as bridges for the induction of similar studies in other wild species.

Functional-structural plant modeling is an invaluable tool for the study of root phene integration. *SimRoot* will continue to be of great utility in this endeavor, as will other root simulations such as RootMap (Diggle, 1988; Dunbabin, 2007) and R-SWMS (Javaux et al., 2008). Simulations allow the exploration of trait function beyond what is possible in greenhouse and field studies. Genetic and physiological constraints may make it difficult or impossible to study some phene state combinations, but they can still be modeled. Simulations also allow many different climates, soil types, and nutrient levels to be studied. While only contrasting and extreme phene states may be combined factorially for study in the field or greenhouse due to space and labor limitations, modeling allows a greater phenotypic range and phene combinations to be studied. In an iterative fashion, simulations help focus empirical experimentation on the most interesting phenes and phene interactions, while data from empirical studies parameterize and refine root models (Wullschleger et al., 1994). A recent review of three-dimensional root models highlights the various models’ strengths and weaknesses, and proposes how to advance the field by
encouraging wider adoption of root models and by making models more realistic through the inclusion of more explicit plant regulatory networks and soil microorganisms (Dunbabin et al., In Press). Simulations should be integrated with phenomic databases to predict functional implications of phenotypic variation, just as models of predicted gene function and subcellular protein targeting augment genomic databases.

**Future Prospects**

The understanding of phenotypic integration requires research comparing multiple states of single phenes in isolation and in combination, generating phene-function landscapes for multiple environments. Understanding the interaction of phenes is particularly important because there may be emergent properties that cannot be predicted from their function in a single phenotypic background. The phenome is the interface of the genome and the environment. Phenomes and phenotypes arise through plant development under genetic control as influenced by the environment, so genetic information is useful in understanding phenotypic variation. At the same time, we need to know how phenes influence plant function in specific environments, which will require the collaboration of plant biologists, soil scientists, and climatologists. Many phenes will not be under single gene control, so the use of single gene mutants for phene studies may limit inquiry to the presence or absence of a particular phene, but we also need to know how variation in phene states contributes to different aspects of plant function. The use of emerging technologies in plant genetics, such as RNA interference, may allow more complex developmental manipulation through changes in expression levels of several genes that could possibly give rise to ranges of phene states in common genetic and phenotypic backgrounds (Katoch and Thakur, 2013).

Phenes are a property of the organism which has been neglected in the genomic era. The organism is the fundamental biological unit of organization for studies of phenes and phene interaction. It is surprising how little research focuses on organisms *per se*, in contrast to the organism being treated primarily as a tool to understand genes or ecosystems. Organisms are the entities on which natural and artificial selection act, which genes influence, and of which ecosystems are composed (Lewontin, 1970). The variation in phenes embodied within a taxon cannot simply be averaged to generate an ideal individual because this variation has functional and evolutionary importance. Progress in understanding the plant genome is stunning, and currently far
outstrips our understanding of the plant phenome, despite the fact that the plant phenome is at least as complex as the genome and arguably more important for human welfare.

The study of phenes is hindered by the lack of relevant conceptual frameworks. Here we have discussed phenes in the traditional context as building blocks of an organism’s phenotype. In some cases it may not be clear whether a phene is truly elemental, as it may be influenced by other traits at lower levels of organization. For example, basal root number in common bean was found to be influenced by basal root whorl number (Miguel, 2012). However, the discovery of even more elemental phenes is a useful outcome of applying the phene perspective. The ambiguity of the phene might be necessary for it to be applied in diverse fields and research programs, but the science of the phenome, phenes and phene interactions will be aided by the development of more precise and informative theoretical frameworks. A better understanding of integrated phenotypes would have benefits for other fields of biology and agriculture, such as how natural selection has led to the diversity of forms observed within and among species, and how improved crop varieties can be designed and developed. Trait-based, or ideotype, breeding is an important avenue for crop improvement, and has been shown to be more efficient than yield-based selection in some situations (Annicchiarico and Pecetti, 1998). Yield and metrics closely associated with yield, such as number of grains, may obscure the advantages of phene states that happen to be in otherwise poor backgrounds. Genetic and developmental pathways may overlap among quantitative traits such as root phenes, so genetic associations with yield or other functional responses are also of limited use. Phene utility should be measured in the field, and for specific environmental stresses, because the advantages of some phene states may only reveal themselves when resources are limiting. Understanding the functional utility of specific root phenes and their interactions requires the employment of near-isophenic plant material in the field and simulation modeling. The opportunities created by the ability to understand the fitness landscape of integrated ideotypes will eventually lead to greater understanding of ecosystem structure and function, and to superior crop lines bred for specific agricultural contexts.

Alleviation of world hunger despite a burgeoning human population, continually degrading natural resources, and global climate change is a primary human challenge for the 21st century. New crop lines with superior soil resource acquisition will be a valuable tool to that end (Lynch, 2007b; Lynch and Brown, 2012). At the same time, understanding how root phenes influence community structure and ecosystem function will inform governmental policy to curb anthropogenic effects on the climate and environment. Clarification and refinement of phene integration theory, simulation and field studies of phenes and phene interactions, and the
distribution of results and plant materials are all essential for the success of this unprecedented opportunity to deploy phenes to provide solutions for pressing world problems.

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Figure 2-1. Studying the characteristics of phenotypes of different individuals allows us to identify phenes and their existent states. The phenome is the total possible phenotypic potential of a taxon, including all possible phene states. The phenotypes presented here do not represent all possible phenotypes of this phenome.
Phenes and their interactions influence plant functions such as nutrient acquisition, utilization, and carbon economy. In turn, these functions affect agricultural performance measures such as shoot biomass and nutrient content. Ultimately, all these lead to yield (or fitness). Yield is far removed from base functions, which themselves can be multi-tiered and reciprocating. The original diagram was made by Arnold (1983) and reworked for plant ecology by Violle et al. (2007). Here we present it for a phene-centric view in agriculture.
Figure 2-3. A phene-function response curve shows the influence of a single continually varying phene on a plant functional response. A phene may have a linear effect on the response (A), asymptote (C), or have an optimum at middle states (C).
Figure 2-4. (A) Black lines depict a simplified root system with a lateral root on each side of a tap root. The left side has 4 second order laterals, while the right side has 8 second order laterals. The darkest grey area around roots depicts the depletion zone of immobile resources (like P), while the medium grey depicts the depletion zone of mobile resources (like N), and the lightest grey represents very mobile resources (like water). (B) Efficiency is shown by the quotient of the area (pixel counts) of a respective resource’s depletion zone divided by the area of the roots for each half of the root system with sparse or dense second order laterals. Dense laterals increase the efficiency for an immobile resource, but decrease efficiency for mobile resources. Differences would be inflated if areas were converted to volumes.
Figure 2-5. Panel A shows the functional response landscape of two phenes that have linear effects in isolation. Panel B shows one phene with a linear effect and one with a central optimum. Panel C shows two phenes with optiums at middle phene states. Synergisms are shown by responses greater than the additive, while antagonistic effects are shown as being less than the additive.
Figure 2-6. A maize seedling is depicted. Seminal roots (blue) and primary root (green) emerge from the seed. One whorl of nodal roots (red) is shown emerging from belowground stem tissue. The nodal roots on the left have steep growth angles, while those on the right are shallow. The shallow nodal roots on the right also have dense laterals, while the steep nodal roots on the left have sparse laterals. In the context of phosphorus acquisition from the epipedon, shallow nodal roots with many laterals will have a synergistic interaction because they are acting within the same module. Though the seminal roots on the left have many laterals they will not interact synergistically for foraging with nodal root traits because they are in a different root class module. The whole plant is integrated by reciprocal signaling between shoot and roots and by balancing the production of photosynthates with soil resource acquisition.
Figure 2-7. Phene integration of root cortical aerenchyma (RCA) and crown root (CR) number was studied in maize using SimRoot across a range of nitrogen (N) and phosphorus (P) levels. These simulation results demonstrate linear, asymptotic, and optimum single phene responses and their interactions.
Figure 2-8. Long root hairs and shallow basal root angles interact synergistically on phosphorus acquisition in the field (created from Miguel, 2012).
Table 2-1. Classification of Root Phenotypes. Classification of a particular root phene begins by determining its mechanism affecting resource uses, acquisition or utilization. Resource acquisition phenes are classified based on their foraging strategy, exploration or exploitation for a particular resource, with nitrogen (N) representing mobile and phosphorus (P) representing immobile resources. All root phenes are classified by whether they influence metabolic economy or are neutral.

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<th>Root Phene</th>
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<td>Axial Root Growth Angle</td>
<td>Acquisition</td>
<td>Exploration</td>
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<td>Root Growth Rate</td>
<td>Acquisition</td>
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<td>Number of Axial Roots</td>
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<td>Lateral Root Branching</td>
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<td>Rhizosphere Modification</td>
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<td>Root Etiolation</td>
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Chapter 3 Spatiotemporal variation of nitrate uptake kinetics within the maize (Zea mays) root system is associated with greater nitrate uptake efficiency but few interactions with root system architecture

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Abstract

Food production must double by 2050 in order to feed the predicted population of nine billion people. Increasing maize nutrient acquisition efficiency, particularly for nitrate, provides one opportunity for increasing food production without using more land, and also for decreasing environmental pollution caused by the leaching of nitrate from agricultural systems. Nitrate uptake kinetics, determined by $I_{\text{max}}$ and $K_m$, have been studied for 50 years, yet significant knowledge gaps remain in both the benefits of possible changes in kinetics, and the metabolic costs associated with the kinetics. Nitrate uptake kinetics have been studied predominantly at the molecular level and the whole root system level, with little regard for how nitrate uptake kinetics may vary within the root system and the functional ramifications of this variation. We used a novel method to measure the nitrate uptake kinetics of short root segments from maize and demonstrate variation of kinetic parameters of nitrate uptake among different root classes of the maize root system. $I_{\text{max}}$ varied among root class, plant age, root axis position, and nitrate deprivation time combinations, but was most affected by plant age, which increased $I_{\text{max}}$, and nitrate deprivation time, which decreased $I_{\text{max}}$. $K_m$ was greatest for crown roots relative to seminal and lateral roots. We used the structural-functional simulation model \textit{SimRoot} to demonstrate the sensitivity of uptake to both $I_{\text{max}}$ and $K_m$. 
and to study the interaction of $I_{\text{max}}$ with root system architectural phenes. Simulated plant growth was more sensitive to $I_{\text{max}}$ than $K_m$, and plant growth generally reached an asymptote near the maximum $I_{\text{max}}$ observed in the empirical studies. Increasing the $I_{\text{max}}$ of lateral roots had the largest effect on shoot growth relative to other root classes. Little interaction was observed between $I_{\text{max}}$ and architectural phenes, which highlights the need to understand the structural and metabolic costs of increasing $I_{\text{max}}$ and to include those costs in simulations. Phenotyping lateral root $I_{\text{max}}$ in genome wide association studies may provide valuable insights in how $I_{\text{max}}$ is determined by genetic and developmental processes, while field studies comparing maize lines contrasting in $I_{\text{max}}$ may provide new insights to its functioning in soil.

Introduction

An increase of at least 100% in food production is necessary to meet the requirements of the nine billion people predicted by 2050 (Royal Society, 2009) to address global food insecurity, a defining challenge of this century (Funk and Brown, 2009). Nearly one billion people are already experiencing hunger (Godfray et al., 2010), so the challenge is imminent. Farming more land is not a viable solution for this problem (Pretty, 2008), so land use efficiency must increase dramatically. Optimization of crop nutrient acquisition efficiency is an important method with which to produce food more effectively (Lynch, 1998), especially because in much of the developing world, use of nitrogen fertilizer is limited (FAO, 2008). In developed nations, intensive nitrogen fertilization pollutes water and the atmosphere (Jenkinson, 2001). Global maize yield is greater than any other grain crop and maize is grown on 177 million Ha (FAO, 2012), and is important for both subsistence and commercial agriculture. Greater nitrogen acquisition efficiency in maize would dramatically improve worldwide agricultural production and potentially mitigate environmental risks.

Nitrogen uptake, or acquisition, efficiency is usually calculated as the proportion of nitrogen available in a field that a plant accumulates in its shoot at maturity (Moll et al., 1982). Nitrate is generally the most abundant available form of nitrogen in agricultural systems and acquired by crops in the greatest amounts (Miller and Cramer, 2004). The rate of nitrate absorption by root tissue is determined by nitrate uptake kinetics, and this rate influences total nitrogen acquisition. Epstein and Hagen (1952) first reported the use of Michaelis-Menten (MM) kinetics to describe root uptake of nutrients. Uptake kinetics were modeled as an uptake rate that saturates as
the nitrate concentration in solution surrounding the roots increases. Given these assumptions, the relationship between uptake rate and external nitrate concentration is summarized with the Michaelis-Menten parameters $I_{\text{max}}$ and $K_m$ (see equation 5). $I_{\text{max}}$ is the maximum influx rate of nitrate, $K_m$ denotes the external nitrate concentration at which half of $I_{\text{max}}$ is obtained, and $C_{\text{min}}$ is the minimum external nitrate concentration at which uptake may occur. These parameters can be measured at different levels of organization.

Research on nitrate uptake kinetics has occurred at three distinct levels of biological organization: transporters, root segment, and root system. However, research integrating across these levels is rare. The most basic level is the transporter level, which has mainly focused on the identification of nitrate transporters (Quaggiotti et al., 2003, 2004; Tsay et al., 2007; Trevisan et al., 2008), and more recently the molecular basis of nitrate uptake (Parker and Newstead, 2014). The intermediate level is at the scale of a root segment, a short section of root where many transporters in the epidermis, along with their interactions with other cellular processes, collectively determine the uptake of nitrate from solution for the root segment (Lazof et al., 1992; Sorgonà et al., 2011). Root segments collectively form a total root system which integrates all roots to generate plant level nitrate uptake through its interaction with soil (Pace and McClure, 1986; Hasegawa and Ichii, 1994). Variation of nitrate uptake kinetics among root segments will determine how root segments influence total root system uptake, along with their interaction with the spatiotemporal distribution of nitrate concentration in soil, which varies greatly (Beckett and Webster, 1971). Variation for uptake kinetics within the root system is likely determined by root class and age.

The transporters responsible for the shuttling of nitrate from external solution (soil or otherwise) have been elucidated. A high affinity (low $K_m$) transport system (HATS) and a low affinity (high $K_m$) transport system (LATS) have been discovered, with transporter proteins encoded by the $NRT1$ and $NRT2$ gene groups, respectively, in Arabidopsis (Tsai et al., 2007). In maize, $ZmNrt1$ and $ZmNrt2$ genes correspond to differences in uptake relating to expression levels (Quaggiotti et al., 2003, 2004; Trevisan et al., 2008). The nitrogen kinetics of a root segment will partially be a function of the relative abundance of each of these transporters, and since they are divided into the HATS and the LATS, $K_m$ and $I_{\text{max}}$ may vary independently at the root segment level.

Uptake kinetics have primarily been measured on whole root systems (Pace and McClure, 1986; Hasegawa and Ichii, 1994) with little regard to possible differences among or within root classes, or on excised roots (Rao et al., 1997), which introduces complications due to the wound response. In maize, differential $^{15}$N accumulation was demonstrated for the primary root tip, other
zones of the primary root, and the primary root laterals, but neither $I_{\text{max}}$ nor $K_m$ were reported (Lazof et al., 1992). In another case, $I_{\text{max}}$ and $K_m$ were determined along intact maize primary roots using a compartmented chamber, but no other classes were included (Sorgonà et al., 2011). To our knowledge, nitrate uptake kinetic parameters have been phenotyped across many maize genotypes in only one study (Pace and McClure, 1986) which determined $I_{\text{max}}$ and $K_m$ for 15 lines at the whole root system level (averaged by total root dry weight). Better understanding of the relationship between transporter properties and abundance integrating to determine uptake kinetics at the level of root segments, and how root segments interact within the whole root system to determine whole plant uptake in the context of the dynamics of soil nitrate bioavailability is necessary before variation in uptake kinetics can be deployed in plant breeding.

Another important contributor to nitrogen acquisition efficiency is root system architecture, which is important in agricultural systems (Lynch, 1995; Ho et al., 2004; Hirel et al., 2007) and natural systems (Mahall and Callaway, 1992; Comas and Eissenstat, 2009) because of its effects on soil resource acquisition, plant interactions, and nutrient cycling. The identification of root phenes (i.e., basic units of the phenotype, sensu Serebrovsky, 1925), and understanding how root phenes influence soil resource acquisition are critical for crop improvement (Kell, 2011; Lynch and Brown, 2012). The maize root system is comprised of an embryonic root system that emerges from the seed, and as the plant grows, successive whorls of nodal roots that emerge from the shoot (Hochholdinger, 2009). Many root system architectural phenes influence water and nutrient uptake and root distribution in maize, including crown root number (Saengwilai et al., 2014), topsoil foraging (Zhu et al., 2005), crown root angle (Trachsel et al., 2013), and lateral branching (Zhu and Lynch, 2004; Postma et al., 2014a). These phenes may influence competition and facilitation among plants (Postma and Lynch, 2012). While uptake kinetics will determine the potential rates of nitrate uptake, root system architecture will determine root placement in relation to soil nitrate availability, so kinetic and architectural phenes likely interact (York et al., 2013) to form an integrated phenotype (see Figure 1).

Here we report a novel method for measuring uptake kinetics from intact root segments within a whole root system using individual segment-specific chambers, how uptake kinetics differ among root classes and ages in a maize cultivar, and simulation results demonstrating how kinetics influence plant performance and interact with root system architecture. The functional utility of spatiotemporal variation of nitrate uptake kinetics within a root system is discussed within the framework of integrating research of nitrate uptake kinetics across the levels of biological organization and implications for natural and agricultural systems.
Materials and Methods

Empirical measurements of N uptake kinetics

Seeds were germinated, as described below, but with two groups staggered 5 days apart as to eventually have both 15 day and 20 day old plants at the time of uptake measurements. For 20 day old plants, plants were deprived of nitrate, as described below, for either 2 days or 5 days before the uptake measurements at 20 days.

Maize (*Zea mays* L. Dekalb DKC44-92) seeds were germinated on germination paper soaked in 0.5 mM CaSO\(_4\) in a dark incubator at 28°C for three days. The seedlings were then transplanted to 30 l hydroponics containers with 9-12 plants per container. The nutrient solution contained 1.5 mM Ca(NO\(_3\))\(_2\), 0.5 mM K\(_2\)SO\(_4\), 0.25 mM Ca(H\(_2\)PO\(_4\))\(_2\), 0.5 mM MgSO\(_4\), 75 µM Fe-DTPA (diethylene triamine pentaacetate). A few grains of Fe(NH\(_4\))(SO\(_4\))\(_2\) salt were added weekly to prevent leaf iron deficiency symptoms), and micronutrients (Hoagland and Arnon 1950). The pH was adjusted to 5.5 using KOH. The hydroponic solution was aerated using two aquarium stones attached to an air pump. The containers were placed in the greenhouse with additional light provided by a set of sodium halide bulbs to maintain 16 hours day length. The remains of the pericarp and endosperm were removed nine days after germination. The nutrient solutions were changed every week. Depending on the experiment, the plants were transferred to a NO\(_3\)-free nutrient solution for nitrate deprivation, where Ca(NO\(_3\))\(_2\) was replaced by CaSO\(_4\), 2 or 5 days before measurement of uptake kinetics.

Four 15 d old or three 20 d old plants, depending on the experiment, were transferred to the lab in 0.5 mM CaSO\(_4\) + 0.5 mM K\(_2\)SO\(_4\) solution (the procedure solution), to which 150 µM KNO\(_3\) was added for induction of the nitrate transport system (Hole *et al.*, 1990). This aerated solution was changed every hour for 6 hours. During this time, the plant leaves were illuminated by a 100 W sodium-halide bulb, which provided 103 µmol m\(^{-2}\) s\(^{-1}\) PAR at leaf level. The plants were then transferred to a 40x25 cm bath, which contained 2 or 3 liters of the procedure solution for 15 d and 20 d plants, respectively, at 25°C, with aeration. The roots were covered with a sheet of aluminum foil to avoid exposure to direct light. KNO\(_3\) was added to the bath to provide initial nitrate concentration between 5-150 µM on different runs.

Ten minutes later, 4 cm long segments of 6.3 mm (1/4 in) inner diameter polyvinyl chloride (PVC) pipe were mounted on the target root sections: 0-4 cm (tip + elongation zone) or 4-8 cm
(maturation zone) of the following root classes: seminal, crown, first-node brace, and laterals of the seminal roots (Figure 2). The tubes included a small 3 mm port in the middle covered with a drop of silicone sealant (Silicone II*, GE, Huntersville, NC, USA) that would later allow samplings of the inner solution with a syringe. The tube and encapsulated root were submerged in the nutrient solution which allowed solution to completely fill the tube with no air bubbles through the open ends. Then, the tubes were completely sealed on both ends with high-vacuum silicon grease in order to isolate the root segment from the solution bath. The solution in the bath was sampled several times during the tube loading to determine the initial concentration ($C_0$, see Eq. 1). After one hour, the root on both sides of the tube was cut, the tube was removed from the bath solution and its contents retrieved with a syringe. The root chamber solution samples were stored in 6 ml vials and immediately frozen. The samples were analyzed for final nitrate concentration ($C_t$, see Eq. 1) using ion-chromatography (Dionex ICS-1100). The root sections were stored in 25% ethanol, and their length and mean diameter were determined using WinRhizo Pro software (v. 2002c, Regent Instruments, Canada).

**Michaelis-Menten Calculations**

Influx to the target root section may be calculated as:

$$I_n = -\frac{V(C_t - C_0)}{A(t - t_0)} \quad \text{equation 1}$$

where $I_n$ is mean net influx to the root segment, $C_0$ is the initial nitrate concentration of the bulk solution at mounting time ($t_0$) and $C_t$ is the nitrate concentration within the tube at sampling time ($t$); $A$ is the absorbing surface area of the root segment; and $V$ is the volume of the solution in the tube. The negative sign in Eq. (1) accounts for the negative concentration change with time of the solution as being opposite to the positive influx to the root, assuming that uptake by the root is the only sink for nitrate.

The root length that was actually exposed to the inner solution is uncertain (see Figure 2), because the grease sealing on both sides occupied unknown volume of the tube. The exact volume of the solution is therefore also unknown. However, the volume $V$ of the solution in the tube equals the internal volume of the tube minus the volume of the grease sealing and the volume occupied by the root. Taking $L$ as the effective root length exposed to the solution, and $r$ as the root radius, and assuming the root length to match to that of the void:
\[ V = \pi L (R^2 - r^2) \quad \text{equation 2} \]

and
\[ A = 2\pi r L \quad \text{equation 3} \]

where \( R \) is the inner radius of the tube and assuming cylindrical geometry of both the tube and the root. Substituting \( V \) and \( A \) in Eq. (1) with those of (2a) and (2b) yields:
\[ I_n = -\frac{(c_t-c_0)(R^2-r^2)}{2\pi r(t-t_0)} \quad \text{equation 4} \]

Equation (3) includes the measured concentrations at the start \((t_0)\) and at the end \((t)\) of the depletion trial, the radius of the tube and that of the root. The uncertain values of the effective root length exposed to the solution and of the actual volume of the solution are not necessary, as they are expressed by measurable or provided parameters: the radius of the root may be accurately determined for a homogeneous short sections of cylindrical, young roots by the WinRhizo Pro software and that of the tube is given. Using units of \( \mu \text{mol cm}^{-3} \) for the concentrations, \( cm \) for the radii and \( s \) for time will result in net-influx in \( \mu \text{mol cm}^{-2} s^{-1} \).

The influx data were plotted against mean initial nitrate concentration, from which the Michelis-Menten (M-M) kinetic coefficients were calculated by non-linear curve fitting (Siddiqi et al. 1990):
\[ I_n = \frac{I_{\text{max}}(C - C_{\text{min}})}{K_m + (C - C_{\text{min}})} \quad \text{equation 5} \]

where \( I_n \) is net-influx to the root, \( C \) is concentration, and \( I_{\text{max}}, K_m \) and \( C_{\text{min}} \) are parameters standing for maximal influx, concentration when \( I_n=0.5 \ I_{\text{max}} \) and concentration where \( I_n=0 \), respectively.

**Structural-Functional Plant Modeling in SimRoot**

In order to investigate the integration of nitrogen kinetics and root system architecture, the functional-structural plant model SimRoot was used (Lynch et al., 1997; Postma and Lynch, 2011a). For detailed information on the structure and function of SimRoot, readers are referred to Postma and Lynch (2011a) and (2011b), but the most pertinent details will follow. SimRoot simulations include both a starting seed and soil conditions, and the soil defined by soil, water, and nitrate properties. The seed produces root axes with properties parameterized by extensive empirical research, except for properties manipulated for the simulation experiment. In this study, all plant properties remained the same in all simulations except for nitrate kinetics and architecture.
parameters as described below. The model includes a non-spatially explicit canopy model with expansion of leaf area leading to increased photosynthesis, and with growth rates constrained by maximums measured in real plants. Maximum growth rate is slowed proportionally as nitrogen stress increases, and nitrogen stress also increases the relative carbon allocation to the root system. The soil transport model \textit{SWMS\_3D} (Simunek \textit{et al.}, 1995) is used to simulate water and solute movement in the soil, such that root uptake results in loss of water and nitrate from the soil which will drive water and nitrate flux in the soil. The simulated soils include parameters affecting water and nitrate movement and include mineralization of nitrate from organic matter.

First, sensitivity analysis of the whole maize root system to Imax and K_m was conducted by varying them independently of each other, with all classes of roots having the same values of Imax and K_m. Imax was varied across 9 levels between 6 and 70 pmol cm\(^{-2}\) s\(^{-1}\). K_m was varied across 9 levels between 5 and 80 \(\mu\)M. For both Imax and K_m, the range selected includes values slightly less and greater than the observed minima and maxima from the empirical component of this manuscript (see Table 1). In order to test the effect of variation for Imax among root classes, we held Imax constant at 6 pmol cm\(^{-2}\) s\(^{-1}\) for all root classes except independently increased Imax to 46 pmol cm\(^{-2}\) s\(^{-1}\), which was near the maximum observed empirically, for lateral, seminal, crown, and brace root classes. In all cases, nitrogen availability was varied between 20 to 200 kg ha\(^{-1}\) across 5 levels, which corresponds to initial soil solution nitrate concentrations between 250 \(\mu\)M and 2500 \(\mu\)M.

Architectural phene states that increase root length density would be expected to increase the overlap in nitrate depletion zones that are extended by increases in Imax, thereby decreasing any benefit Imax would have in the absence of increased inter-root competition. All levels of Imax were factorially combined with 4 levels of nodal root number, ranging between 8 and 46 nodal roots, which represents the range observed in maize recombinant inbred lines in the field (Trachsel \textit{et al.}, 2011). All levels of Imax were factorially combined with 4 levels of nodal root angle, ranging between 20 and 80 degrees from horizontal, or from very shallow to very steep, which represent the range observed in the field (Trachsel \textit{et al.}, 2011). All levels of Imax were factorially combined with 5 levels of lateral root branching, ranging between 2 and 20 laterals cm\(^{-1}\), which represents the range observed in the field (Trachsel \textit{et al.}, 2011). In all cases, nitrate availability was varied between 20 to 200 kg ha\(^{-1}\) across 5 levels. The importance of Imax during interplant competition was evaluated by simulating two plants either with the same Imax (intraphenotypic competition) or with different Imax (interphenotypic competition), with the two levels of Imax being 46 pmol cm\(^{-2}\) s\(^{-1}\) and 6 pmol cm\(^{-2}\) s\(^{-1}\), which represent the maximum and minimum values, respectively, observed in the empirical experiments. All simulations had two replicates and standard error was less than 1% of
the mean in all cases because SimRoot is fundamentally a deterministic model, with variation caused by small random changes to growth angles at each time step.

**Results**

**Empirical**

I$_{\text{max}}$ varied both among root classes, root position, plant ages, and number of days of nitrate deprivation (Table 1, Figure 2), with the slowest I$_{\text{max}}$ being 14.02 pmol cm$^{-2}$ s$^{-1}$ observed in the 0-4 cm region of crown roots at 15 days of age after 2 days of nitrate deprivation, and the greatest I$_{\text{max}}$ being 46.52 pmol cm$^{-2}$ s$^{-1}$ observed for crowns in the 4-8 cm region at 20 days of age after 2 days of nitrate deprivation. On average, there were no significant differences in I$_{\text{max}}$ among root classes, although differences exist at some positions, age, and deprivation levels (Table 1). In general, position along a root axis did not have a large or a consistent effect on I$_{\text{max}}$. I$_{\text{max}}$ increased 93% from 20.358 pmol cm$^{-2}$ s$^{-1}$ to 39.362 pmol cm$^{-2}$ s$^{-1}$ from 15 to 20 day old plants, respectively (p = 0.00196). The only general trend for K$_{\text{min}}$ was being consistently lower for seminal and lateral roots compared to crown roots (p = 0.00268), with an average of 11.9 µM for seminal and lateral roots and an average of 36.5 µM for crown roots. In 5 of 6 cases, 5 day nitrate deprivation led to slow uptake relative to 2 day deprivation and a linear relationship between external nitrate concentration and uptake.

**Simulation**

The empirical data described above were used to parameterize SimRoot to compare the effects of varying nitrate kinetics on uptake and the interactions of kinetics with RSA. Sensitivity analysis for I$_{\text{max}}$ (Figure 3) showed that increasing I$_{\text{max}}$ increased shoot mass, but generally shoot mass reached an asymptote by 40 pmol cm$^{-2}$ s$^{-1}$, which was near the maximum value observed empirically. In the lowest level of nitrogen, shoot dry mass increased 54% from the lowest to highest value of I$_{\text{max}}$, while at the highest level of nitrogen, there was a 183% increase. The response to increasing I$_{\text{max}}$ is made more complex by the simulated plant’s response to stress, such that the shoot mass response to increasing I$_{\text{max}}$ fluctuates. Sensitivity analysis for K$_{\text{m}}$ (Figure 4)
demonstrated less effect on plant performance across all N levels than did I_{max}, with only an 8% increase in shoot dry weight at the lowest level of nitrogen (20 kg N ha^{-1}), comparing the greatest value of K_m to the least. At the second most severe level of nitrogen stress (40 kg N ha^{-1}), there was a 12% increase in shoot dry weight associated with decreasing K_m.

The I_{max} dependency for a specific root class (Figure 5) was demonstrated by holding all other root classes to a slow I_{max}, 6 pmol cm^{-2} s^{-1}, while increasing the I_{max} of the focal root class to the greatest empirically observed I_{max}, 46 pmol cm^{-2} s^{-1}. Shoot dry weight was most dependent on lateral root I_{max} followed by seminal, crown, and brace root classes. The utility of I_{max} for shoot growth will depend on the phenotypic background in which it exists, so we modeled its interaction with three root system architectural phenes: nodal root number (Figure 6), nodal root angle (Figure 7), and lateral root branching (Figure 8). In general, there was relatively little interaction between I_{max} and the architectural traits, such that increasing I_{max} generally increased shoot growth regardless of the root system architectural context in which it was expressed. On average, the range of shoot growth influenced by I_{max} was greater than the range of shoot growth as influenced by root system architecture.

At the lowest level of nitrogen (20 kg N ha^{-1}), plants had less shoot mass in the sandy soil with high leaching than in the clay soil (Figure 9). Soil type did not influence the general trend of increasing I_{max} benefitting plant growth, but growth in sandy soil did tend to shift the local optima to greater values of I_{max}. Under conditions of interphenotypic competition with plants with high and low I_{max} grown together, high I_{max} plants had 15% more shoot mass, while low I_{max} plants grew 9% less shoot mass relative to their shoot masses during intraphenotypic competition (Figure 10).

**Discussion**

Nitrate uptake kinetics varied among root classes, with I_{max} being greatest for lateral and crown roots and K_m being least for lateral and seminal roots. Variation for nitrate uptake kinetics among root classes has not previously documented for several root classes and ages. Older plants had greater I_{max} and similar K_m regardless of root class. Plants deprived of nitrate for more days before uptake measurements had slower uptake, and a linear response rather than a saturating response, possibly because deprivation leads to low internal concentrations and allows passive influx of nitrate (Siddiqi et al., 1990). Older plants have a greater demand for nitrogen, which may explain their increased I_{max}, because greater I_{max} would allow greater uptake. Plants deprived of
nitrogen will reduce their growth rate, so the decreased $I_{\text{max}}$ of nitrate deprived plants may relate to the decreased demand of the plant. These results showing that plant nitrogen demand relates to nitrate kinetics is consistent with other reports (Garnett et al., 2013). The linear response of the nitrate deprived plants may relate to the plant having a greater reliance on the low affinity transport system, which is known to have a linear response (Glass et al., 1992; Touraine and Glass, 1997) possibly because of passive uptake in a channel-like state when cytoplasmic nitrate concentration is low (Wang et al., 2012), which may especially be true in the case of more nitrate deprived plants. Lateral roots had greater $I_{\text{max}}$ than their parent roots, possibly because lateral roots dominate total root system length and are responsible for the majority of nutrient uptake, as confirmed in the simulation component. The differences among root classes and plant ages demonstrate that spatiotemporal variation of nitrate uptake kinetics within the root system is an important phenomenon in need of further characterization.

In the simulations, $K_m$ exhibited relatively less effects on shoot mass than $I_{\text{max}}$, but increases in shoot mass of 10% in stressful soils at 40 days of growth does represent a potential opportunity, especially because this increased growth will compound over time. Increasing $I_{\text{max}}$ was associated with more than a doubling of shoot mass in some simulations. Increasing $I_{\text{max}}$ had a complex effect on shoot mass at lower N levels because greater values of $I_{\text{max}}$ allowed nitrate to be acquired in sufficient amounts, which decreased plant stress during early plant growth. SimRoot increases the relative allocation of carbon to the root system compared to the shoot when the plant experiences nitrogen stress, and decreases the relative allocation to the root system when stress is alleviated (Postma and Lynch, 2011a). However, this stress response may not always optimize plant growth because root growth is irreversible and compensating with new growth is a slow process (Postma et al., 2014b). A greater $I_{\text{max}}$ value allows a plant to uptake adequate nitrate during early growth so relatively less mass will be allocated to the root system. As the shoot grows and demands more nitrogen, the smaller root system cannot meet this demand even at the greater $I_{\text{max}}$ value, so the plants becomes stressed again and photosynthesis can’t maintain shoot growth. However, in many cases if $I_{\text{max}}$ is increased further this stress can be alleviated by the increased N uptake per root length, however all simulations end at 40 days so plants are at different levels of nitrogen stress and compensation through root growth. This behavior is difficult to predict and exaggerated when interacting with phenes that influence carbon economy, such as nodal root number and lateral root branching when compared to a carbon neutral phene like nodal root angle, which had a smoother response. In the simulation model ROOTMAP, plasticity of nitrate uptake kinetics was found to contribute greatly to the uptake of herringbone (sparsely branched) type root systems, but with little
contribution to total nitrate uptake of dichotomous (greatly branched) type root systems in simulations where nitrate supply was heterogeneous (Dunbabin et al., 2004). In general, greater $I_{\text{max}}$ should have more benefit when combined with phene states that decrease overall root system density, such as decreased nodal root number, decreased lateral branching, and moderate rooting angles.

The Barber-Cushman model (Barber and Cushman, 1981; Barber, 1984) provides the interface between the root surfaces and nutrients found in the soil water in SimRoot. Barber (1984) previously described sensitivity analysis of several of the model parameters for nitrate uptake, however the original Barber-Cushman model assumes equidistance between roots which ignores root system architecture and assumes the soil is homogeneous with regards to nutrient concentrations. Still, reviewing those results is informative. In the original sensitivity analysis for nitrate uptake from the Barber-Cushman model, nitrate uptake was particularly sensitive to the growth rate of roots, $I_{\text{max}}$, and the root radius (Barber, 1984). The model was scarcely influenced by the mean root distance (root density) or the initial concentration of nitrate. The model was completely insensitive to $K_m$. Barber’s sensitivity analysis had a high initial nitrate concentration which explains the linear response of nitrate uptake to increasing $I_{\text{max}}$, and this relation did not reach an asymptote as in the current SimRoot model. The $I_{\text{max}}$ used in Barber’s analysis was derived from whole root system uptake of maize in a silt loam soil, and was equivalent to 10 pmol cm$^{-2}$ s$^{-1}$, so even when doubled as part of the sensitivity analysis the asymptotic point of around 40 pmol cm$^{-2}$ s$^{-1}$ was not reached. The $K_m$ used by Barber was 25 µM, in the mid-range of that used here, so the complete insensitivity in the Barber model was because of the high nitrate concentrations and short duration, whereas in the SimRoot simulations at low nitrogen or after uptake of most of the available nitrate, $K_m$ can have a small effect on nitrate uptake. The current simulations contribute to the literature by demonstrating the importance of nitrate kinetics parameters for specific root segments and by demonstrating additive effects with root system architecture.

This study focused on variation of nitrate uptake kinetics among root classes and ages and how this variation affects total root system uptake. The demonstration of spatiotemporal variation in kinetics implies considerable developmental and genetic controls through unknown processes that must affect the relative abundances of different types of transporters and other processes affecting nitrate uptake, as discussed below. The use of transgenic mutants with transporter gene insertions and knockout mutants would not be appropriate for documenting and understanding natural variation of intra-root system uptake kinetics and its functional utility because such mutants have only binary functional states (on or off) and are mostly useful for confirming the role of a
gene in a functional process. This research will benefit from the screening of multiple genotypes for these phenes. We expect root segment uptake kinetics to have complex, quantitative control because of the integration of many other phenes, as discussed below.

Functional-structural plant modeling is an invaluable tool for the study of the functional utility of root system phenes (Dupuy et al., 2010), including root uptake kinetics and interactions with other root phenes. Root system simulation models that include nutrient uptake such as SimRoot, ROOTMAP, SPACSYS, R-SWMS, and RootBox (reviewed in Dunbabin et al., 2013) will be of great utility in the study of the functional ramifications of changes in nitrate $I_{\text{max}}$ and $K_m$. Simulations allow the exploration of uptake kinetics and their interactions with other plant phenes in more combinations of climates, soil types, and nutrient levels than is possible in greenhouse and field studies, due to labor and financial constraints. Genetic and physiological constraints may make it difficult or impossible to study some phene state combinations, but they can still be modeled. In an iterative fashion, simulations allow physiologists to target their experimentation on phenes and phene interactions that show the most promise, and the information gained from empirical studies contribute to simulation work (Wullschleger et al., 1994). The lack of strong interactions between nitrate kinetics and root architectural phenes in this study may be affected by a lack nitrate uptake metabolic costs, such as protein synthesis and osmotic regulation, which is a knowledge gap discussed more below. Including these costs in simulation models will be an important contribution to understanding utility of nitrate uptake kinetics for total root system nitrate uptake.

Understanding nitrate uptake kinetics must occur within the broader context of ecological interactions. Physiological plasticity of nitrate uptake kinetics may be a method for plants to quickly respond to patches or pulses of nitrate before roots are able to proliferate through branching and growth (reviewed by Hodge, 2004). During competition, plants with greater $I_{\text{max}}$ may acquire more nitrate than their competitor, as demonstrated in this study’s simulation component. Despite construction costs of transporters and energetic costs associated with nitrate uptake, acquiring resources before a competitor may increase relative fitness and answer the question of ‘why plants bother’ to proliferate roots and increase uptake kinetics (Hodge et al., 1999). In another simulation study, nitrate uptake kinetics ranked highly among many root and soil properties for their influence on crop-weed competition (Dunbabin, 2007). Increasing fitness relative to competitors is important in natural systems, but can lead to a ‘tragedy of the commons,’ a prediction of game theory where plants over-proliferate roots relative to the optimal amount of roots to maximize uptake efficiency (Gersani et al., 2001). However, evidence for the ‘tragedy of the commons’ with regards to roots is conflicting (Semchenko et al., 2007; Dudley and File, 2007; Nord et al., 2011). In contrast,
avoidance of root growth redundancy, or over-proliferation, has been hypothesized to be important for agriculture systems where optimizing yield of the focal crop is the goal (Zhang, 1999). Similarly, considering the costs of transporter construction and uptake energetics, there may be greater transporter redundancy and uptake costs when optimizing relative fitness in natural systems than in agricultural systems where nutrient uptake efficiency may be more important.

$I_{\text{max}}$ has been known to be an important factor influencing nitrate uptake for 50 years (Lycklama, 1963; Rao and Rains, 1976; Siddiqi et al., 1990). However, to our knowledge $I_{\text{max}}$ has never been a target of a breeding program, and significant knowledge gaps remain in understanding the functioning of $I_{\text{max}}$ across biological levels of organization. Root segment $I_{\text{max}}$ is a phene aggregate (see York et al., 2013) influenced by more fundamental processes. Understanding nitrate uptake necessitates formalizing the relationship between the uptake observed for a root segment on per length, area, or weight basis, and the kinetics observed for the respective individual transporters. Nitrate kinetic values of root segments are necessarily phene aggregates influenced by the number and types of nitrate transporters in the epidermis of a root segment. Although the relationship between root segment $I_{\text{max}}$ for nitrate and the number of nitrate transporters is not known, a linear 1:1 relationship between transporter surface density and overall uptake was found for another transporter (Garcia-Celma et al., 2013). Root segment $I_{\text{max}}$ may be equal to the combined $I_{\text{max}}$ of all individual transporters of various identities. Recently, expression of $NRT2$ from Chrysanthemum in Arabidopsis resulted in the doubling of nitrate uptake in hydroponics, while additionally expressing $NAR2$ from Chrysanthemum resulted in a quadrupling of nitrate uptake in hydroponics (Gu et al., 2014). The number of nitrate transporters is related to transcription levels as well as post-transcriptional and post-translational processes (Wirth et al., 2007; Gu et al., 2014) so understanding the regulation of transporter generation is imperative for understanding how nitrate kinetics are determined at the root segment level. Root segment $K_{m}$ must be influenced by the relative abundance of HATS and LATS transporters, possibly the weighted average of constituent transporter $K_{m}$ values based on abundance. More research is needed to clarify how absolute number of the various nitrate transporters and their relative proportions are regulated by gene redundancy, transcription levels, and post-transcriptional and post-translational processes.

Ultimately, however, $I_{\text{max}}$ and $K_{m}$ of transporters occur at the molecular level, and we do not know what specific properties of the transporters are responsible for variation in transporter $I_{\text{max}}$ and $K_{m}$. Variation in transporter kinetics may exist as influenced by gene variants, or alleles, about which we know little. Parker and Newstead (2014) suggest that phosphorylation of a specific residue within $NRT1.1$ allows greater flexibility and, so, greater nitrate uptake. However, in that
case, the same phosphorylation event may interfere with the nitrate binding site and increase the $K_m$ (Parker and Newstead, 2014). If so, we have reasonable evidence that modifying or selection of transporters may be possible for greater uptake rates and binding affinities, or that natural variation in uptake parameters might exist at the transporter level. The energetics of the secondary active transport process for nitrate uptake must also be considered: given the stoichiometry of the plasma membrane H+ ATPase proton pumping (Sze et al., 1999) and nitrate transporter uptake (Parker and Newstead, 2014), every nitrate ion absorbed requires at least 1 ATP molecule to maintain the proton gradient. Veen (1980) determined that the respiration required for nitrate uptake accounted for 20% of total plant respiration. The construction cost of transporters may be estimated based on their protein structure and abundance, as well as the respiration required for their synthesis and shuttling to the epidermis external membrane. As described above, understanding the construction and maintenance costs of transporters along with the costs of uptake energetics is necessary to inform simulation modeling for optimizing uptake kinetics in whole root systems, and to understand competitive dynamics in natural and agricultural systems.

Several approaches are needed in order to use nitrate kinetics phenes in breeding programs. Expression of varying levels of different nitrate transporters in different proportions must be related to nitrate uptake at higher levels of biological organization, such as per cell membrane area. High-throughput phenotyping approaches may be used for measurements of this phene aggregate at the root segment level method used in the current study. Phenotyping of root segment nitrate kinetics coupled to genome wide association studies could prove to be a very powerful approach to quickly discover many genomic regions associated with optimal kinetics and to use those in breeding programs. Since lateral roots have the highest uptake rate and comprise the majority of the maize root system, lateral roots would be a sensible target of root segment nitrate kinetics high-throughput phenotyping. Increasing nitrate uptake efficiency and optimizing kinetics based on knowledge of nitrate transporters have long been proposed as method to transform agriculture. In the simulations, plant growth was more influenced by $I_{\text{max}}$ than $K_m$ in realistic virtual soils, so $I_{\text{max}}$ may be a more important focus of future research. The optimal $I_{\text{max}}$ will be defined as the point where the marginal benefit equals the marginal cost (Bloom et al., 1985), and both benefits and costs associated with increasing $I_{\text{max}}$ have significant knowledge gaps. Leveraging high-throughput phenotyping, simulation modeling, genomic analysis, and laboratory molecular research together will allow agricultural scientists to realize the promise of increasing nitrate acquisition efficiency and provide one component of the solution to the challenge of global food insecurity.
References


Figure 3-1. A SimRoot rendering of a typical maize root system that integrates nitrate kinetics and root system architecture. The simulation is at 40 days of age and is colored by $I_{\text{max}}$ where warmer colors indicate greater $I_{\text{max}}$. In this configuration axial roots have greater $I_{\text{max}}$ than laterals, and the growing tips have decreased $I_{\text{max}}$ relative to the more mature non-elongating roots segments.
Figure 3-2. Schematic of the tube chamber for measuring nitrate depletion rate due to absorption by a non-excised root segment. The port in the top middle was sealed, an intact root was inserted through the tube, the tube submerged in the controlled nitrate solution, then sealed on both ends with silicon sealant such that the enclosed root was surrounded by nitrate solution. Dimensions needed for calculation of Michaelis-Menten kinetics are: L – the length of tube between the two sealant caps; R – the inner radius of the tube; and r – the radius of the root. These terms are used to calculate influx to the root, see Eq. (3) in the text.
Figure 3-3. Nitrate influx at varying concentrations of nitrate in seminal, lateral, crown, and brace root classes of maize. Nitrate influx is compared between 15 day old (15d) and 20 day old (20d) plants deprived of nitrate for 2 days before measurements, and between 20 day old plants at either 2 days of nitrate deprivation (2d) or 5 days of nitrate deprivation (5d). Points represent individual observations, lines represent fitted Michaelis-Menten models, and bands represent 90% confidence intervals.
Figure 3-4. In order to conduct $I_{\text{max}}$ sensitivity analysis on shoot growth, maize plants were simulated with a range of 9 $I_{\text{max}}$ values growing in soils at 5 nitrogen levels using SimRoot. The range of $I_{\text{max}}$ includes values smaller and greater than those observed in the empirical component of this study. The color of the line indicates the nitrogen level in which the simulations grew and are smoothed with loess for ease of interpretation.
Figure 3-5. In order to conduct $K_m$ sensitivity analysis on shoot growth, maize plants were simulated with a range of 9 $K_m$ values growing in soils at 5 nitrogen levels using SimRoot. The range of $K_m$ includes values smaller and greater than those observed in the empirical component of this study. The color of the line indicates the nitrogen level in which the simulations grew and are smoothed with loess for ease of interpretation.
Figure 3-6. In order to test the dependency of shoot growth on the $I_{\text{max}}$ of specific root classes, maize plants were simulated using SimRoot with variation in the $I_{\text{max}}$ of different root classes across 5 levels of nitrogen. $I_{\text{max}}$ was held constant for all root classes at 6 pmol cm$^{-2}$ s$^{-1}$, which was near the minimum observed, except that a focal root class was independently increased to 46 pmol cm$^{-2}$ s$^{-1}$, which is near the maximum observed. The color of the line indicates the focal root class that had increased $I_{\text{max}}$ grew and are smoothed with loess for ease of interpretation. The control simulations have all root classes set to the slower $I_{\text{max}}$. 
Figure 3-7. In order to test the interaction of $I_{\text{max}}$ and nodal root number (NRN), simulations of maize were conducted varying $I_{\text{max}}$ across 9 values with root systems with 8, 22, 34, or 46 nodal roots. Results are shown for 2 levels of nitrogen, 20 kg N ha$^{-1}$ (left) and 40 kg N ha$^{-1}$ (right). The color of the line indicates the NRN of the simulations and are smoothed loess lines for ease of interpretation.
Figure 3-8. In order to test the interaction of $I_{\text{max}}$ and nodal root angle (NRA) simulations of maize were conducted varying $I_{\text{max}}$ across 9 values with root systems with NRAs of 20, 40, 60, or 80 degrees from horizontal (larger number is more steep). Results are shown for 2 levels of nitrogen, 20 kg N ha$^{-1}$ (left) and 40 kg N ha$^{-1}$ (right). The color of the line indicates the NRA of the simulations and are smoothed loess lines for ease of interpretation.
Figure 3-9. In order to test the interaction of $I_{\text{max}}$ and lateral root branching density (LRBD), simulations were conducted varying $I_{\text{max}}$ across 9 values with root systems with LRBDs of 2, 5, 10, 15, or 20 lateral roots cm$^{-1}$. Results are shown for 2 levels of nitrogen, 20 kg N ha$^{-1}$ (left) and 40 kg N ha$^{-1}$ (right). The color of the line indicates the LRBD of the simulations and are smoothed loess lines for ease of interpretation.
Figure 3-10. The results of the interaction between $I_{\text{max}}$ and nodal root number (NRN) are shown for the lowest level of nitrogen, 20 kg N ha$^{-1}$, in two soils, a clay-loam soil (Clay) and a sandy-loam soil (Sand) that differ primarily in that the sandy soil has greater nitrogen leaching.
Figure 3-11. The results of competition between plants with the same $I_{\text{max}}$ (intraphenotypic) or different $I_{\text{max}}$ (interphenotypic). The high $I_{\text{max}}$ was 46 pmol cm$^{-2}$ s$^{-1}$ and low $I_{\text{max}}$ was 6 pmol cm$^{-2}$ s$^{-1}$, which represent the maximum and minimum values observed in the empirical experiments, respectively.
Table 3-1. Michaelis-Menten kinetics coefficients calculated for nitrate influx to intact roots of corn grown in hydroponics for 15 or 20 days, deprived 2 or 5 days prior to the determination procedure. In each column, values with the same letter are not significantly different at $p \leq 0.05$ levels according to the paired t-test. Combinations where net-influx ($In$) responds linearly to the concentration ($C$) are represented by the linear regression.

<table>
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<th>Age (d)</th>
<th>Deprivation (d)</th>
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<th>Position† (cm)</th>
<th>$I_{max}$ (pmol cm$^{-2}$ s$^{-1}$)</th>
<th>$K_m$ (µM)</th>
<th>$C_{min}$ (µM)</th>
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Chapter 4

Intensive phenotyping of the maize (Zea mays L.) root crown demonstrates substantial intra-crown variation and relationships among root phenes, shoot mass, and nitrogen uptake in low nitrogen soil

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Introduction

Global food security is a preeminent challenge of the 21st century (Funk and Brown, 2009), and food production must increase by at least 100\% to meet the requirements of the 9.5 B people predicted by 2050 (Royal Society 2009; World Bank 2014). The fact that ca. one billion people of the seven billion now living are experiencing hunger (Godfray et al., 2010) accentuates this pressing need. Farming more land is not a viable solution for this problem (Pretty, 2008), so land use efficiency must increase dramatically. Optimizing plant nutrient use efficiencies is one way to more productively use land (Lynch, 1998), especially because in much of the developing world, use of nitrogen (N) and phosphorus (P) fertilizers is negligible (FAO 2008). In developed nations, much of the applied fertilizers are not taken up by plants and eventually pollute water and the atmosphere (Jenkinson, 2001). Maize is grown on 177 million ha, with aggregate yield exceeding all other grains (FAO 2012), and is a mainstay of both subsistence and commercial agriculture. Reducing the fertilizer requirement of maize acquisition by developing genotypes with improved nutrient acquisition is an important goal for both subsistence and commercial agriculture (Lynch, 2007).

Root system architecture (RSA) has important effects on soil resource acquisition, plant interactions, and nutrient cycling in agricultural systems (Lynch, 1995, 2007; Ho et al., 2004; Hirel et al., 2007; Zhang et al., 2014) and natural systems (Mahall and Callaway, 1992; Comas and Eisenstat, 2009). The identification of root phenes (i.e., basic units of the phenotype, sensu Serebrovsky, 1925; Lynch and Brown, 2012; see York et al., 2013 for discussion), and
understanding their utility for soil resource acquisition is an important step in phene-based or ideotype breeding critical for crop improvement (Kell, 2011; Lynch, 2014). The maize root system is comprised of an embryonic root system consisting of the primary root and seminal roots that emerge from the seed, and successive whorls of nodal roots that emerge from the shoot (Hochholdinger, 2009). In the root system architectural taxonomy adopted by the International Society for Root Research, the primary root, seminal roots, and nodal roots of maize would be classified as the tap root, basal roots, and shoot-borne roots, respectively (Zobel and Waisel, 2010), however we use the maize-specific terminology here. Many root architectural, anatomical, and morphological phenes and phene aggregates influence water and nutrient uptake, and root distribution in maize, including crown (belowground nodal) root number (York et al. 2013; Saengwilai et al. 2014), topsoil foraging (Zhu et al., 2005a), crown root angle (Trachsel et al., 2013), lateral branching (Zhu and Lynch, 2004), root cortical aerenchyma (Zhu et al. 2010; Saengwilai et al. 2014), living cortical area (Jaramillo et al., 2013), and root hairs (Zhu et al. 2010). These same phenes may influence competition and facilitation among plants (Ge et al., 2000; Rubio et al., 2001, 2003; Postma and Lynch, 2012; Zhang et al., 2014). Understanding how these root phenes interact with one another to give rise to a functionally integrated phenotype in different environments is a complex challenge (York et al., 2013). Even though many of these root architectural phenes have been associated with functional utility in the field, little is known about variation of root phenes among the nodes of the maize root system and whether this variation could have functional importance for soil resource acquisition.

Variation of root phenes within the maize root system is difficult to study because the outer whorls of the maize root system are the youngest roots and occlude the older roots in the interior. When root phene differences among nodes are studied only one line may be reported (e.g. Picard et al. 1985), or else an incomplete measurement of only a few nodes is conducted for many lines (e.g Guingo et al. 1998). One approach to increase throughput of field phenotyping is the use of digital images of excavated root crowns combined with automatic image analysis (Grift et al., 2011; Bucksch et al., 2014), however the use of mature root systems prevents measurements of the occluded part of the root system, and neither of these image analysis platforms count individual nodal roots, which contributes to the important phene aggregate of nodal root number (NRN). The use of mesocosms containing clear gels has been another approach that allows detailed architectural analysis over time, however growth is limited to young plants and such media may introduce artifacts due to its dissimilarity to field soil (Iyer-Pascuzzi et al., 2010). Micro-CT using X-rays is another promising approach to visualize roots over time in soil, however pot size is currently a
limitation (Mooney et al., 2011). Likewise, measurements of nodal root growth angles in the field have been limited to the outer brace (aboveground nodal) and crown roots (Trachsel et al., 2011), and measurements of angle in containers may not be valid because of the physical constraints of container size. Screening of maize seminal root systems has been conducted extensively on germination paper in the lab (Zhu et al., 2006; Hund et al., 2009) but has not been confirmed by field experiments and does not provide insight about the nodal root system. However, imaging of mature root crowns from the field can be conducted while excising whorls of nodal roots to reveal interior whorls, and nodal roots can be counted manually and angles measured using software.

While progress is being made in relating root phenes to soil resource acquisition in maize, these phenes are commonly measured on only a single whorl or else aggregated across all whorls. In order to test the hypotheses that root architectural phenes are influenced by the node on which they manifest, that variation in root architectural phenes within the root system can be as great or greater than variation observed among genotypes, and that phene states of all whorls contribute to nitrogen uptake and plant growth we intensively phenotyped the root crowns of several maize lines under two nitrogen levels in the field.

Materials and Methods

Similar field experiments were conducted in Pennsylvania in the United States in 2012 and in Limpopo province in South Africa in 2014 (details below). Because overall results were similar between the two sites and to provide more detail in the main text, the results from South Africa will be primarily presented in the main text, while all data will be provided for the United States in supplemental material.

Plant Material

Twelve recombinant inbred lines were used from the intermated B73 x Mo17 (IBM) population (Sharopova et al., 2002) with genotypes listed in Supplemental Table 1 along with experimental identity. These 12 RILs were originally selected to have contrasting nodal root growth angles.
**Experimental Site**

The field site was near Alma, Limpopo province, South Africa (24°33’ 00.12 S, 28°07’25.84 E, 1235 masl) with the soil being a Clovelly loamy sand (Typic Ustipsamment). The average temperature between planting and sampling was 22° C, total rainfall was 340 mm, and average relative humidity was 70%.

**Treatment Installation**

The experiment included 4 blocks within a 20 ha center pivot irrigated field. Each field was split in half randomly to create split-plots in which to create a high nitrogen (HN) and a low nitrogen (LN) soil environment. Entire fields received phosphorus (P) and potassium as determined by soil tests. A planter passed through all fields leaving behind non-planted rows in which to plant manually. Within the HN and LN split-plots, IBM lines were random assigned to plots. Each plot contained 5 rows that were 4 meters long with a row distance of 76 cm. Seeds were hand-planted on November 23, 2013 into rows marked by the planter using stakes and ropes marked to accommodate a density of 80,000 plants ha⁻¹. At planting, 23 kg N ha⁻¹ was applied to the entire field through center pivot fertigation, and an additional 23 kg N ha⁻¹ was supplied as granular urea in the HN split-plots. HN split-plots received 46 kg N ha⁻¹ as granular urea approximately every 3 weeks to reach a total of 184 kg N ha⁻¹. Additional water was supplied as needed with center pivot irrigation. Micronutrients were supplied at a rate of 3 kg ha⁻¹ 60 days after planting.

**Experimental Sampling and Harvest**

Root crowns were sampled from two blocks each day between February 4-5, 2014 (72-73 DAP). Three entire plants were excavated and processed in the field from each plot using the shovelomics method (Trachsel et al., 2011) with the shovel inserted 30 cm from the base of the plants. The three root crowns were soaked in water then rinsed with a water hose and nozzle until most soil was removed. One representative root crown was selected for subsequent architectural measurements based on its apparent average size and uniformity.
Root Crown Imaging and Architectural Measurement

The original shovelomics method (Trachsel et al., 2011) was modified to accelerate field processing while permitting more intensive measurements. Root crowns were kept in large plastic bins submerged in water inside a 5º C cold room until they were imaged within one week. Root crowns were imaged using digital cameras attached to frames with camera mounts such that the camera was facing down from a height of 50 cm. Three identical cameras (PowerShot A1200, Canon, Melville, NY, USA) operated by three researchers were used to process samples quickly. Root crowns were placed under the camera on a matte black background. A 3 cm white plastic disk was included as a scale in every image, along with a printed sample label. Camera zoom and focus were kept locked for the duration of the imaging. An image was taken of every whorl of nodal roots (Figure 1) by removing all roots in a node sequentially. A representative nodal root was excised from the side of the root crown not facing the camera for each whorl and placed to the side of the root crown such that both root crown and representative nodal root were in frame of the image.

Image analysis was conducted in RSAJ1 which is a project for the ObjectJ plugin (Vischer and Nastase, 2009) for ImageJ (Schneider et al., 2012). RSAJ prompts the user to take sequential measurements from the images (Fig. 2), which are briefly described (see RSAJ manual for elaboration). Nodal root angle from horizontal was derived trigonometrically from the stem width, maximum root crown width, and the height between stem width and root crown width. The number of nodal roots counted for a whorl was multiplied by 2 in order to account for the occluded half of the root system, based on previous observations of symmetry in maize root crowns. The diameter of the representative nodal root was measured, along with the distance from the basal cut to where lateral roots emerge (distance to branching). In order to calculate lateral root branching density, the number of lateral roots was counted along a measured length on the representative nodal root. Finally, the lengths of three representative lateral roots were measured and averaged for analysis. Distances in pixels were converted to cm or mm by using the pixel width of the circular 3 cm scale. See Table 1 for a list and of all measured root phenes and their abbreviations. Images were renamed prior to analysis with all experimental information and node position. Node position was labeled in the order of development, with position 1 being the oldest coleoptilar node (Hund et al., 2011).

1 Available at: http://plantscience.psu.edu/roots/methods/computer/RSAJ
Statistical Analysis

All statistics were conducted and data graphics were created with R version 3.0.2 (R Core Team, 2013). ANOVA was conducted with mixed effects modeling using nlme with nitrogen level nested within block as the random effect and nitrogen level, genotype, and node position as the fixed effects (Table 2). In order to quantify the proportion of variation in root phenes contributed by the node position and genotype, the effect size $\eta^2$ was calculated from ANOVA sums of squares (Table 3). Linear regressions were conducted separately for HN and LN between each root phene in each whorl individually against shoot mass, percent nitrogen in the leaves, and total leaf nitrogen content with data from all genotypes. Phenes with regression p-values less than 0.1 were combined for multiple regression and then stepwise regression was used to find the most parsimonious model predicting shoot mass, percent nitrogen, and total nitrogen from root phenes using the step function in R. Principal component analysis (PCA) was conducted on averages of the 4 replicates for nitrogen level, genotype, and node combinations (n = 165, incomplete rows omitted) using the princomp function. Root crown phene aggregates were derived from the raw data, including total nodal root number and the number of nodes for each root crown and analyzed by t-test for a nitrogen effect. Graphs were created in R with the ggplot2 package (Wickham, 2009).

Results

Influence of node position, genotype, and N level on phene values (Table 1)

Stem width, averaged across N level and genotype, generally increased from .41 cm in the first node to ~ 1.5 cm in the last nodes (Fig. 3, p < .01). Among genotypes, stem width, averaged across node positions and N levels, ranged between .83 cm and 1.15 cm (p < .01). Stem width decreased from 1.06 cm to .89 cm from HN to LN, respectively (p < .01). All interactions were significant. The node position explained 71.3% of the variation in stem width, while genotype explained 3.1% (Table 3).

Nodal root growth angle (NRGA) from horizontal was affected by node position and ranged between 51.7° and 54.8° in the first three whorls and between 60.2° and 65.4° in the last 6 whorls, averaged across genotype and N level (Fig. 4, p < .01). Among genotypes, averaged across node position and N level, NRGA ranged between 54.2° and 62.0° (p < .01). The effect of N level,
overall, was not significant. Generally, the angle increased (became steeper) from 1st to 4th node position, and from the 4th position some genotypes increased angle, some remained the same, and others decreased angle (significant interactions). The node position explained 17.3% of the variation in NRGA, while genotype explained 6.5% (Table 3).

Nodal occupancy (NO) increased with the node position, from an average of 4 roots in the first whorl to an average of 11 roots in the last whorl (Fig. 5, p < .01). Average nodal occupancy among genotypes ranged between 5.3 and 6.8 roots (p < .01). Average nodal occupancy decreased from 6.2 in HN to 5.5 in LN (p < .038). The rate of change and total number of roots differed among genotypes, where some produced 4 roots on the first 3-4 whorls before increasing, while others began producing more roots earlier (node x genotype significant interaction). The node position explained 53.6% of the variation in NO, while genotype explained 2.28% (Table 3).

Nodal root diameter (NRD) increased with node position from 1.0 mm in the first whorl to ~4.5 mm in the last whorls (Fig. 6, p < .01). Among genotypes, averaged across node position and N level, NRD ranged from 2.7 mm to 3.2 mm (p < .01). NRD decreased from 3.0 mm in HN to 2.7 mm in LN (p = .015) Generally, the increase with node position was linear, though in some cases diameter leveled off with the transition from crown to brace roots around the 6th node position. The node position explained 80% of the variation in NRD, while genotype explained 1% (Table 3).

The distance to branching (DTB), over average, increased from .56 cm in the first node to 2.0 cm in the last node (Fig. 7, p < .01). However, not all genotypes exhibited this increase with node position (significant genotype x node interaction). DTB among genotypes, averaged across node positions and N levels, ranged from .40 cm to 1.2 cm (p < .01). DTB decreased from .95 cm in HN to .70 cm in LN (p = .027). The node position explained 12.5% of the variation in DTB, while genotype explained 10% (Table 3).

Lateral root branching density (LRBD), on average, increased with node position from 7.3 to 13.0 lateral roots cm⁻¹ in the first and last nodes, respectively (Fig. 8, p < .01), although this increase doesn’t occur for all genotypes (significant node by genotype interaction). Among genotypes, averaged across node position and N level, LRBD ranged from 6.9 to 10.9 lateral roots cm⁻¹ (p < .01). N level had no significant effect on LRBD. The node position explained 11.7% of the variation in LRBD, while genotype explained 14.9% (Table 3).

Lateral root length (LRL), on average, increased with the node position from 0.64 cm in the first node to 3 cm in the last node (Fig. 9, p < .01). Among genotypes, averaged across node position, LRL ranged between 1.25 cm and 2.44 cm (p < .01). N level had no significant effect on
LRL. The node position explained 22.6% of the variation in LRL, while genotype explained 7.3% (Table 3).

**Influence of nitrogen on root crown phene aggregates nodal root number and number of nodes**

Nodal root number (NRN), the combined number of nodal roots in an entire root crown, ranged between 31 and 53.75 among genotypes and nitrogen levels (Fig. 10). Nodal root number decreased 16% from 44 to 36 from HN to LN, respectively (p < .01). The total number of nodes within a root crown varied between 5.7 and 8.5 among genotypes and nitrogen levels (Fig. 10). The number of nodes averaged across genotypes decreased 6% from 7 to 6.6 nodes from HN to LN, respectively (p = 0.02159). The linear models predicting nodal root number from number of nodes in both HN (y = 6.09x + 0.92, p < 0.01) and LN (y = 4.82x + 4.49, p < 0.01) were significant. Correlations between total number of nodal roots and the occupancy of each whorl generally showed relationships between adjacent whorls. In LN, the occupancies of whorls 2 and 6 were most correlated with NRN [Supplemental Information Fig. 1]. In HN, the occupancies of whorls 3, 5, and 7 were most correlated with NRN [Supplemental Information Fig. 2].

**Principal component analysis**

Principal component analysis of the average phene values for nitrogen level, genotype, and node position combinations revealed two principal components, PC1 and PC2, that explain 60% and 16% of the total variation, respectively (Fig. 11). PC1 was primarily influenced by nodal root diameter, lateral root length, stem width, and nodal occupancy. PC2 was primarily influenced by distance to branching, lateral root branching density, and nodal root growth angle. The scores of PC1 are heavily dependent on node position, where younger whorls have greater PC1 scores. Correlational analysis supports the structure of these components, where nodal root diameter, lateral root length, stem width, and nodal occupancy are strongly correlated with each other and with the node position [Supplemental Information Fig. 3].
Relations among root phenes, shoot mass, and N content

In LN, linear regression of all root phenes from all whorls against shoot mass identified 24 phenes with significant relationships. Stepwise regression of the most significant root phenes of different whorls in LN, and not including stem widths, revealed a model containing +LRBD.1, +NRGA.3, +NRGA.4, +DTB.4, +NRGA.5, and +NO.5 (numeric suffix denotes the node position, + and – indicating positive and negative relations, respectively) as the most parsimonious model which accounted for 69% of the variation in shoot mass (Fig. 12). In HN, linear regression of all root phenes from all whorls against shoot mass identified 22 phenes with regression p-values less than 0.1. Stepwise regression of the most significant root phenes of different whorls in HN, and not including stem widths, revealed a model containing +LRD.1, -NRGA.2, +LRL.4, and +LRL.5 as the most parsimonious model which accounted for 49% of the variation in shoot mass (Fig. 13). In LN, a multiple regression model including the nodal occupancies of all whorls explained 34% of shoot mass variation, while a regression model with total nodal root number explained 22%. In HN, neither the multiple regression model of all whorl occupancies nor the regression model with NRN were significant. Percent reduction in shoot mass was calculated for every genotype and block combination, then all root phenes were regressed, which identified 12 root phenes with regression p-values less than 0.1. Stepwise regression of these root phenes identified -NRGA.4, -NRD.5, and -NRN as the most parsimonious model explaining 33% of the variation in percent reduction in shoot mass (p < .01).

Linear regression of percent nitrogen in leaves from plants grown in LN soil as a response to all root phenes in all whorls identified 14 phenes with regression p-values less than 0.1. Stepwise regression of the most significant root phenes of different whorl, not including stem widths, revealed a model containing -LRL.2, +NRD.3, and -LRBD.6 as the most parsimonious model which accounted for 36% of the variation in leaf percent nitrogen (p < .01). In HN, no root phene had a significant effect on percent leaf nitrogen. The results of regressions of total leaf nitrogen content were largely driven by the effect of leaf mass and similar to the shoot mass results above so are not included. Log transformations of the data did not substantially influence the results and most relationships appear linear.
Discussion

This study presents a novel analysis of the variation of several root architectural phenes both within and among maize root crowns for several genotypes in high and low nitrogen soils and how root architectural variation affects performance in low nitrogen soils. The most sensitive phenes to node position were size-related phenes such as stem width, nodal occupancy, and nodal root diameter, and these phenes tended to have greater variation within root crowns than among root crowns. However, most phenes had considerable variation both within and among root crowns, especially distance to branching and lateral root length. Nodal root growth angle had the least variation, both within and among root crowns. Nodal root number and the number of nodes varied greatly among genotypes and nitrogen levels, and both were decreased by nitrogen level. There was a significant positive correlation between the number of nodes and the nodal root number. Principle component analysis demonstrated that most variation is explained by size-related phenes and the first principal component could discriminate node position. In general, relationships among root phenes of different whorls and among root phenes and shoot properties such as mass and percent N demonstrate the importance of measuring the root phenes of all whorls. More phenes correlated with shoot mass of plants grown in low nitrogen soil than in high nitrogen soil, especially NRGA of several whorls, and these phenes were different from the phenes that correlated with shoot mass of plants grown in high nitrogen soil. The differing relationships of phenes with biomass in LN and HN suggests the relationships are not allometric (i.e. are not inherently related to plant size, Niklas 2004). Stepwise multiple linear regression suggests that the additive integration of several phenes, each with small effects, can explain a large amount of the variation observed in shoot mass, in this case almost 70%.

Most studies measuring maize root architecture in the field focus on the outer whorls of brace and crown roots (Trachsel et al., 2011, 2013; Grift et al., 2011; Bucksch et al., 2014), though these whorls, especially brace, arguably contribute the least to soil resource acquisition because they emerge late and into zones of soil already explored by other roots. This study demonstrates that a more detailed analysis of the root system is necessary for physiological studies quantifying the functional utility of specific root phenes. Phenes measured at the node level may be more elemental and useful for genetic studies as well, supported by the lack of constant relationships among phene states among nodes in different genotypes. Recently, intensive phenotyping of lateral root phenes of several orders were accomplished using semi-automated methods in a single hybrid of maize (Wu and Guo, 2014). Dissection of the root crown also allows the counting of nodes,
which could be an important phene because of its relation to total nodal root number and the internal resource balance of the plant. However, this method does not allow more than a basic understanding of the temporal dynamics of node emergence which was shown to be important in simulations (York, 2014). Weekly sampling doing similar RSA measurements may be important for determining variation in the emergence times of nodes. Earlier work has documented the increase in nodal occupancy with node position (Picard et al., 1985; Hoppe et al., 1986; Stamp and Kiel, 1992) but differences among genotypes were not reported nor was the number of nodes described as an independent root phene of maize previously.

Fewer crown roots were recently demonstrated to increase N uptake, shoot mass, and yield in low nitrogen soils using recombinant inbred lines of maize (Saengwilai et al. 2014), confirming earlier simulation results (York et al., 2013). In this study, a positive relationship between NRN and shoot mass existed for plants grown in low nitrogen soil, but not for plants grown in high nitrogen soil, which suggests the relationship in LN was not necessarily allometric because variation existed for NRN and shoot mass in HN but they were not related. One important distinction between the current study and the previous study of Saengwilai et al. (2014) is that Saengwilai et al. selected genotypes that performed equally well in HN, which was not necessarily the case in this study. In this case, more nodal roots may be associated with greater vigor, especially when integrated with other varying root phenes. This study offers several other novel insights to the potentially important NRN phene aggregate (defined as a property combining several more elemental phenes, such as nodal occupancies and the number of nodes). In LN soil, NRN was dependent mostly on the occupancies of the 2nd and 6th whorls, so intensive phenotyping of this phene aggregate by decomposition into more elemental phenes such as nodal occupancies for every whorl could benefit breeding programs. A multiple regression model of shoot mass including the linear combinations of the nodal occupancies of every whorl was more explanatory than NRN alone, which again demonstrates the importance of more intensive phenotyping. Selecting for fewer root nodes as a first round of breeding may facilitate eventual selection for elite maize with fewer nodal roots, and has the benefits of being both simple and fast.

In many genotypes used in this study, nodal root growth angle were steeper in younger whorls, which agrees with earlier reports (Feldman, 1994). However, in other genotypes NRGA was almost the same in all whorls or else were more shallow in younger whorls. Studies of a single maize genotype are often extrapolated to the species level, yet here and elsewhere substantial variation for almost any measured root phene was demonstrated so genotype-to-species level extrapolations are generally not warranted. Measuring NRGA on only outer whorls is not a reliable
way to predict the overall pattern of growth angles of whorls of nodal roots within a maize root crown. In this study, in LN soil steeper angles of older and younger whorls were associated with greater shoot mass, while in HN shallow roots in whorl 2 were slightly associated with greater shoot mass. In this experiment, N was only applied in LN plots one time then leached deeper, while N was applied several times in HN plots so constituted a generally shallow resource. Plasticity of nodal root growth angle has previously been observed in maize, with shallow-angled genotypes in HN becoming up to 18º steeper in LN (Trachsel *et al.*, 2013). Steep-angled nodal roots have been hypothesized to benefit maize plants grown with deficits of deep resources such as leaching nitrate or water during terminal drought (Lynch, 2013), while shallow roots of maize are known to increase phosphorus uptake (Zhu *et al.*, 2005a) and possibly uptake of other shallow resources.

Increased lateral root branching density of the first whorl was associated with increased shoot biomass in both the low and high nitrogen soils of this study, and ranged between about 4 and 10 lateral roots cm\(^{-1}\). Recently, simulation studies concluded that between 6 and 10 lateral roots cm\(^{-1}\) optimize nitrate acquisition depending on the nitrate levels with substantial declines in plant dry weight beyond 10 lateral roots cm\(^{-1}\) (Postma *et al.*, 2014). The same simulation study concluded that at least 10 lateral roots cm\(^{-1}\) optimize phosphate acquisition, with no decline in plant dry weight if lateral root branching density is increased further. The maximum of about 10 lateral roots cm\(^{-1}\) in the first whorl found in this study could suggest co-optimization of both nitrogen and phosphorus acquisition during early growth. Early plant vigor is an important characteristic of maize ideotypes (Mock and Pearce, 1975), and in this study the stem width at the first node (a proxy for early growth) was positively correlated with total shoot mass (LN: \(y = 146x - 3.5, r^2 = .18, \ p = .004;\) HN: \(y = 441x - 39.9, r^2 = .24, \ p < .001\)).

In the high nitrogen soils, but not in LN, increased lateral root length of the 4\(^{th}\) and 5\(^{th}\) whorls correlated strongly with plant shoot mass. Long laterals may increase the volume of soil exploited for nitrate without creating substantially more competition among roots of a system, and long laterals are a component of the SCD ideotype (Lynch, 2013). This hypothesis is further confirmed by a simulation study in maize (Postma *et al.*, 2014) where an optimal density of lateral roots allowed carbon and nitrogen investment in longer lateral roots which benefitted nitrate uptake. The coefficient of variation for lateral root length was greater for roots grown under HN than LN for both the 4\(^{th}\) and 5\(^{th}\) whorls, and there was a statistical interaction between nitrogen level x genotype for lateral root length. Some lines in this study exhibited a plastic response to elevated soil nitrogen by which they grew longer laterals which possibly lead to increased N uptake. Even though N was applied several times throughout the season, in the sandy soil used in this study,
longer laterals may especially benefit growth by capturing N before it leaches deeper into the soil. This effect could also be partly allometric, where greater carbon status of the plant leads to greater allocation to laterals. At 80,000 plants ha\(^{-1}\) N, and possibly other unintended resources, can be growth limiting on a per plant basis, and variation did exist for shoot mass even in HN soil. Longer laterals deserve further consideration as a possible breeding target.

The integration of root phenes will determine how multiple phenes interact through their influence on soil resource foraging and plant metabolic status (York et al., 2013). Studying the integration of root phenes in the maize root crown required the intensive phenotyping used in this study in order to understand the root crown as whole constructed of more elemental constituents. Applying a phene-based paradigm necessitates a continual reevaluation of what properties are elemental and developmentally unique (Lynch and Brown, 2012), such as by measuring the properties of individual whorls rather than crown-level aggregates. Ambiguity may exist as to what extent a phene is elemental, but the attempt to clarify the study of the plant phenome will be aided by more precise conceptual definition of phenes and correspondingly, more precise terminology. For example, the number of nodal roots in maize is clearly determined by the number of nodes and the root occupancies of each node, yet we know little about the relations among nodes and whether they are genetically linked such that a more elemental developmental phene could exist that influences the occupancies in each node. The additive multiple linear regression models used in this study show that considering multiple phenes together offers predictive power for understanding plant growth, but phene integration is usually ignored in the study of root phene utility (Ho et al., 2005; Zhu et al., 2005a; Saengwilai et al., 2014b; Chimungu et al., 2014). Multiplicative models of multiple regression (Friedrich, 1982) that included the statistical interactions of phenes were also tested with the current data but had no significant effects and offered no more predictive power, however multiplicative models could offer novel insights in the future for the study of phene interactions that are synergistic rather than additive.

As noted previously, most root phenotyping platforms either screen seedlings on germination paper in the lab, or screen mature root crowns in the field without measuring the occluded, older nodal roots. Direct relations of seedling root phenes to seedling vigor may be an important component of breeding for root system ideotype in crops. Though time consuming, if intensive phenotyping was performed on hundreds of maize lines, genome wide association studies (GWAS, reviewed by Cobb et al. 2013) could be conducted that might identify the genetic basis for phene states that either vary independently or vary dependently among whorls. Studying the development and genetics of roots among whorls could contribute as much insight to the regulation
of root growth as studying the differences among genotypes considering the greater variation of root phenes observed among whorls than among genotypes. Intensive phenotyping may complement extensive phenotyping by targeting the most relevant diversity panels or subsets of lines based upon the results of extensive phenotyping. Wu and Guo (2014) presented novel semi-automated methods to excavate maize root crowns, wash the crowns, measure root phenes, and create visualizations. The extension and improvement of these methods could provide a means to sample large populations. Many imaging methods from gels, to x-rays, to field-based imaging use aggregate measurements or mathematical descriptors of the entire root crown and so obscure the underlying variation within maize root crowns (Iyer-Pascuzzi et al., 2010; Grift et al., 2011; Mooney et al., 2011; Bucksch et al., 2014). However, these same methods could provide the basis of allowing intensive phenotyping to be used extensively on large diversity panels as long as the phenes of different nodes can be measured independently. Recently, a 6 degrees-of-freedom digitizer was used to reconstruct a 3-dimensional model of two maize hybrids grown in the field and to extract information about the curvature of several whorls of nodal roots in maize, which might be another promising method for field phenotyping (Wu et al., 2014). Because of the inherent temporal dynamics of spatial availability of different soil resources, variation within the maize root crown may reflect adaptations to allow maize genotypes to have different spatio-temporal foraging properties. For example, the ‘steep, cheap, and deep’ (SCD) ideotype for optimal water and nitrogen foraging by maize root systems hypothesizes earlier forming axial roots should have many laterals while later forming axial roots should have fewer but longer lateral roots (Lynch, 2013). This study suggests that the decoupling of relationships of phene states among whorls may be possible, but to what extent and benefit is not known.

Near-isophenic plants have similar phenotypic backgrounds and allow the opportunity to compare specific contrasts of phenes without the confounding influences of variation in many phenes. For example, Donald (1962) proposed crossing and backcrossing wheat varieties with horizontal and near-vertical leaves to create near-isogenic and near-isophenic lines with contrasting leaf angles. Recombinant inbred lines (RILs), such as those used in this study, have been used for near-isophenic comparisons of root phenes in common bean and maize (Yan et al., 2004; Zhu et al., 2005b,c, 2006; Ochoa et al., 2006). Biparental RIL populations are useful for these phenotypic contrasts, but also have limited phenotypic diversity which limits their usefulness for studying the utility of root phenes. Structured diversity panels with greater phenotypic variation provide another opportunity for studying root phenes, though more advanced statistics may be needed to account for the influence of variation in several phenes (Mezmouk et al., 2011).
Functional-structural plant modeling will continue to advance the study of root phenes (Dunbabin et al. 2013). Simulations allow more detailed studies of root system functioning than possible in greenhouse and field studies. Simulation models permit the study of phenotypes that do not exist in nature, and many different climates, soils, and soil resource levels can be simulated. Field experiments are costly in terms of land use, labor, and supplies, so simulation modeling allows much larger experimental designs. Simulation modeling informs empirical work, and at the same time empirical work provides the data and insights for new simulations, so modeling and empirical research are synergistic (Wullschleger et al., 1994).

**Conclusion**

Intensive phenotyping of all whorls within the maize root crown revealed that while many size-related root phenes generally increased with younger node positions, other phenes, such as NRGA, DTB, LRBD, and LRL can have different patterns depending on the genotype. Root phenes in older and younger whorls contributed to predictions of shoot mass and percent leaf nitrogen. The interactions of genotype with node position in determining the pattern of root phene states suggests that root phene states may be at least partially independent among nodes. The degree of independence will be important when considering ideotypes as they relate to spatio-temporal soil dynamics (Lynch, 2013) and the integration of root phenes (York et al., 2013). In low nitrogen soil, increased lateral branching density of the first whorl, steeper nodal root growth angles on the 3rd, 4th, and 5th whorls, and more nodal roots on the 5th whorl were associated with greater shoot mass. In high nitrogen soil, increased lateral branching density of the first whorl, more shallow nodal roots on the 2nd whorl, and longer laterals on the 4th and 5th whorls were associated with greater shoot mass. Statistical models accounting for variation in several phenes accounted for a large proportion of the variance in shoot mass. A model including the nodal occupancies of all whorls was found to be a better predictor of shoot mass than total nodal root number alone, and nodal root number was found to be partially determined by the number of nodes. Screening for fewer root nodes in a maize breeding program could be a simple and fast method to initiate a program selecting for fewer nodal roots (York et al. 2013; Saengwilai et al. 2014), providing an example of traditional selection followed by precision selection discussed by Cobb et al. (2013). Intensive phenotyping of both root architectural and anatomical phenes is very rarely attempted, but architectural and anatomical variation in root phenes was described in many lines from different Zea species (Burton...
et al., 2013), and a recent report studied a single maize cultivar and reported several architectural and anatomical phenes for several classes of roots (Gao et al., 2014). Phenotyping that is both intensive and extensive, coupled to GWAS, could be a powerful technique to accelerate the understanding of how the integration of root phenes among whorls affects soil resource acquisition in maize and other crops with potential impacts for global food insecurity.

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References


Figure 4-1. A mature root crown of maize is dissected by excising whorls of nodal roots from the outside to expose the next layer and imaging with a digital camera. Top left depicts brace roots at node position 7, while crown roots at node position 1 bottom, third from right, and the seminal root system, not measured, is at bottom right. A representative nodal root is placed to the side of the root crown in every image, along with a 3 cm plastic disk and a printed label with sample identity. The stem diameter, nodal occupancy, nodal root growth angle, nodal root diameter, distance along the representative nodal root to lateral branching, lateral branching density, and lateral root length were measured for every nodal whorl.
Figure 4-2. Measurements were made with computer assistance using RSAJ, which is available to use with ImageJ. Blue annotations mark root crown measurements, and include stem width (top horizontal line), system width (bottom horizontal line), and the distance between stem and system width (vertical line) for calculating angle with trigonometry, and blue squares count nodal roots, which were counted twice assuming symmetry of the root system. Red annotations mark representative nodal root measurements, including nodal root diameter (top horizontal line), distance to branching (top vertical line), distance along nodal root for counting laterals (middle vertical line), red squares for counting lateral roots, and 3 poly-lines for measuring lateral root length.
Figure 4-3. Stem width was measured at each node position, with position 1 being the oldest whorl. 12 panels are annotated with the identity of the IBM maize genotype. Within each panel, stem width at each node position is colored blue for high nitrogen (HN) and red for low nitrogen (LN). The line height at each node position is the mean of 4 replicates, while vertical lines represent standard error.
Figure 4-4. Nodal root growth angle (NRGA) was measured at each node position, with position 1 being the oldest whorl. Lower values of NRGA indicate more shallow angles. 12 panels are annotated with the identity of the IBM maize genotype. Within each panel, NRGA at each node position is colored blue for high nitrogen (HN) and red for low nitrogen (LN). The line height at each node position is the mean of 4 replicates, while vertical lines represent standard error.
Figure 4-5. Nodal occupancy, or the number of roots in a node, was measured at each node position, with position 1 being the oldest whorl. 12 panels are annotated with the identity of the IBM maize genotype. Within each panel, nodal occupancy at each node position is colored blue for high nitrogen (HN) and red for low nitrogen (LN). The line height at each node position is the mean of 4 replicates, while vertical lines represent standard error.
Figure 4-6. Nodal root diameter was measured at each node position, with position 1 being the oldest whorl. 12 panels are annotated with the identity of the IBM maize genotype. Within each panel, nodal root diameter at each node position is colored blue for high nitrogen (HN) and red for low nitrogen (LN). The line height at each node position is the mean of 4 replicates, while vertical lines represent standard error.
Figure 4-7. The distance to branching (from nodal root base to first lateral emergence) was measured at each node position, with position 1 being the oldest whorl. 12 panels are annotated with the identity of the IBM maize genotype. Within each panel, distance to branching at each node position is colored blue for high nitrogen (HN) and red for low nitrogen (LN). The line height at each node position is the mean of 4 replicates, while vertical lines represent standard error.
Figure 4-8. Lateral branching density was measured at each node position, with position 1 being the oldest whorl. 12 panels are annotated with the identity of the IBM maize genotype. Within each panel, lateral branching density at each node position is colored blue for high nitrogen (HN) and red for low nitrogen (LN). The line height at each node position is the mean of 4 replicates, while vertical lines represent standard error.
Figure 4-9. Lateral length (average of 3 laterals per representative nodal root) was measured at each node position, with position 1 being the oldest whorl. 12 panels are annotated with the identity of the IBM maize genotype. Within each panel, lateral length at each node position is colored blue for high nitrogen (HN) and red for low nitrogen (LN). The line height at each node position is the mean of 4 replicates, while vertical lines represent standard error.
Figure 4-10. A scatter plot and linear regression is shown for the relationship of the total number of nodes in a root crown with the total number of nodal roots in a root crown. Points are the mean of 4 replicates for each genotype, colored blue in high nitrogen (HN) and red in low nitrogen (LN). Blue and red lines indicate the linear model of best fit for HN ($y = 6.09x + 0.92$, $p = 0.0066$) and LN ($y = 4.82x + 4.49$, $p = 0.00266$), respectively.
Figure 4-11. Principal component analysis of root architectural phenes conducted on data averaged across the 4 replicates for each nitrogen level, maize genotype, and node position combination. Points represent the scores of principal components 1 and 2 (PC1 and PC2) for each nitrogen level, maize genotype, and node position combination. Colors of the points indicate the node position, with 1 being the oldest whorl. Labeled lines demonstrate the correlation of phene values to principal component scores (maximum correlation = .9512). Abbreviations are as given in Table 1.
Figure 4-12. Multiple panels show the effect of the most significant and explanatory phenes from all whorls on total shoot mass in low nitrogen plots after stepwise multiple linear regression. Panels A-F demonstrate the relationship of the following phenes with total shoot mass: LRBD.1, NRGA.3, NRGA.4, DTB.4, NRGA.5, and NO.5. Abbreviations are as given in Table 1 and the appended number identifies the whorl in which the phene was measured. Panel G shows fitted values are calculated from the linear combinations of the above phenes using the coefficients determined by multiple linear regression.
Figure 4-13. Multiple panels show the effect of the most significant and explanatory phenes from all whorls on total shoot mass in high nitrogen plots after stepwise multiple linear regression. Panels A-D demonstrate the relationship of the following phenes with total shoot mass: LRBD.1, NRGA.2, LRL.4, LRL.5, NRGA.5, and NO.5. Abbreviations are as given in Table 1 and the appended number identifies the whorl in which the phene was measured. Panel E shows fitted values are calculated from the linear combinations of the above phenes using the coefficients determined by multiple linear regression.
Table 4-1. Phenomes measured within maize root crowns at each node position are listed with their abbreviations (Abbr.), the source of the measurement (from the root crown or the representative nodal root.), and a brief description.

<table>
<thead>
<tr>
<th>Phene</th>
<th>Abbr.</th>
<th>Source</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem Width</td>
<td>SW</td>
<td>Crown</td>
<td>Stem width at a node</td>
</tr>
<tr>
<td>Nodal Occupancy</td>
<td>NO</td>
<td>Crown</td>
<td>The number of nodal root in a node</td>
</tr>
<tr>
<td>Nodal Root Growth Angle</td>
<td>NRGA</td>
<td>Crown</td>
<td>The angle from horizontal in a node</td>
</tr>
<tr>
<td>Nodal Root Diameter</td>
<td>NRD</td>
<td>Root</td>
<td>The thickness at the base of a nodal root from a node</td>
</tr>
<tr>
<td>Distance to Branching</td>
<td>DTB</td>
<td>Root</td>
<td>The length from the base of a nodal root to first lateral</td>
</tr>
<tr>
<td>Lateral Root Branching Density</td>
<td>LRBD</td>
<td>Root</td>
<td>The number of lateral roots from a nodal root in 1 cm</td>
</tr>
<tr>
<td>Lateral Root Length</td>
<td>LRL</td>
<td>Root</td>
<td>The average of 3 lateral roots from a nodal root</td>
</tr>
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</table>
Table 4-2. ANOVA table of maize root phenes giving F-value and significance for all factors and factor interactions. ** p ≤ .01; * .01 < p ≤ .05; ns p > .05, not significant. Phene abbreviations are as in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>SW</th>
<th>NRGA</th>
<th>NO</th>
<th>NRD</th>
<th>DTB</th>
<th>LRBD</th>
<th>LRL</th>
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<tbody>
<tr>
<td>Nlevel</td>
<td>77.87**</td>
<td>0.32ns</td>
<td>12.54*</td>
<td>26.05 *</td>
<td>16.44 *</td>
<td>1.6</td>
<td>7.2</td>
</tr>
<tr>
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<td>13.4</td>
<td>5.32**</td>
<td>3.16**</td>
<td>3.73**</td>
<td>8.21**</td>
<td>12.23**</td>
<td>6.72**</td>
</tr>
<tr>
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<td>156.6**</td>
<td>822.33**</td>
<td>3349.2**</td>
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<td>107.45**</td>
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<td>1.83 *</td>
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<td>3.94**</td>
<td>1.81 *</td>
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<td>2.78**</td>
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<td>1.24ns</td>
<td>1.51ns</td>
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Table 4-3. Effect size as $\eta^2$ demonstrates the amount of variation explained by each factor, interaction, and the residuals $n$ in the ANOVA. Phene abbreviations are as in Table 1.

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<th></th>
<th>SW</th>
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<th>NO</th>
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Chapter 5 Nodal root number and growth angle influence nitrogen and phosphorus acquisition in maize

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Abstract

Increasing the nutrient acquisition efficiency of maize is an important goal for food security and agriculture sustainability. The overall architecture of the maize nodal root system is largely determined by nodal root number (NRN) and nodal root growth angle (NRGA). We hypothesized that NRN and NRGA interact to have substantial effects on soil resource acquisition. We tested this hypothesis in contrasting nitrogen, phosphorus, and plant density scenarios with the functional-structural plant model \textit{SimRoot}. Fewer nodal roots, even zero in some cases, improved growth in soils with moderate to severe nitrogen limitation. For a given NRN, emergence of nodal roots on later appearing nodes improved plant growth. Generally, phenotypes with intermediate NRGA had the greatest growth under moderate to severe nitrogen stress. High population density reduced individual plant growth under nitrogen stress and accentuated the effects of NRN and NRGA. Population density had no effect on phosphorus acquisition. Under moderate to severe phosphorus limitation, the optimal NRN shifted to intermediate values (ca. 15 nodal roots), and phenotypes with shallow NRGA had the greatest shoot mass. Under nitrogen stress the benefit of reduced NRN is driven by reduced intraplant competition and by increased lateral root length, whereas under phosphorus stress some shallow nodal roots are required in order to position root foraging in soil domains with the greatest phosphorus availability. We conclude that maize root systems that are moderately shallow-angled with few nodal roots will optimize plant growth across a range of nitrogen, phosphorus, and density regimes.
Introduction

Global food security is a preeminent challenge of the 21st century (Funk and Brown, 2009), and food production must increase by at least 100% to meet the requirements of the 9.5 B people predicted by 2050 (Royal Society 2009; World Bank 2014). The fact that one billion people of the seven billion now living are experiencing hunger (Godfray et al., 2010) accentuates this pressing need. Farming more land is not a viable solution for this problem (Pretty, 2008), so land use efficiency must increase dramatically. Optimizing plant nutrient use efficiencies is one way to more productively use land (Lynch, 1998), especially because in much of the developing world, use of nitrogen (N) and phosphorus (P) fertilizers is negligible (FAO, 2008). In developed nations, much of the applied fertilizers are not taken up by plants and eventually pollute water and the atmosphere (Jenkinson, 2001). Maize is grown on 177 million Ha, with aggregate yield exceeding all other grains (FAO 2012), and is a mainstay of both subsistence and commercial agriculture. Reducing the fertilizer requirement of maize acquisition by developing genotypes with improved nutrient acquisition is an important goal for both subsistence and commercial agriculture (Lynch, 2007).

Root system architecture has important effects on soil resource acquisition, plant interactions, and nutrient cycling in agricultural systems (Lynch, 1995, 2007; Ho et al., 2004; Hirel et al., 2007) and natural systems (Mahall and Callaway, 1992; Comas and Eissenstat, 2009). The identification of root phenes (i.e., basic units of phenotype, sensu Serebrovsky, 1925; Lynch and Brown, 2012; see York et al., 2013 for discussion), and understanding their utility for soil resource acquisition is an important step in trait-based or ideotype breeding critical for crop improvement (Kell, 2011). The maize root system is comprised of an embryonic root system that emerges from the seed, and successive whorls of nodal roots that emerge from the shoot (Hochholdinger, 2009). Many root architectural, anatomical, and morphological phenes and phene aggregates influence water and nutrient uptake and root distribution in maize, including crown root number (Saengwilai et al., 2014), topsoil foraging (Zhu et al., 2005), crown root angle (Trachsel et al., 2013), lateral branching (Zhu and Lynch, 2004), root cortical aerenchyma (Zhu et al. 2010; Saengwilai et al. 2014), living cortical area (Jaramillo et al., 2013), and root hairs (Zhu et al. 2010). These same phenes may influence competition and facilitation among plants (Ge et al., 2000; Rubio et al., 2001, 2003; Postma and Lynch, 2012). Understanding how these root phenes interact with one another to give rise to a functionally integrated phenotype in different environments is a complex challenge (York et al., 2013).
Maize nodal root phenes such as crown root number and brace root number were proposed to be independent with different optima depending on the soil resource, such as being steep, cheap, and deep to optimize N acquisition (Lynch, 2013). Belowground nodal roots are commonly referred to as ‘crown’ roots while aboveground nodal roots are referred to as ‘brace’ or ‘prop’ roots because they are believed to provide structural support that helps reduce lodging, though evidence for this is surprisingly limited (Liebhardt and Murdock, 1965). Among maize varieties in the Wisconsin Diversity Panel (Hansey et al., 2011), the number of nodal roots ranged between 23 and 79 across two seasons (Eric Nord, unpublished research). Recently, maize genotypes with fewer crown roots were shown to have increased shoot mass, deeper roots, and increased yield in low nitrogen soils relative to plants with more crown roots (Saengwilai et al., 2014). Nitrate is relatively mobile in soil (Schenk and Barber, 1979) so possibly fewer axial roots are needed for adequate foraging since the nitrogen may more readily travel to the root surface through diffusion and mass flow. Roots have large construction and maintenance costs (Veen, 1980) so fewer roots will be especially beneficial for the plant’s carbon balance (Mooney, 1972). However, the number of nodal roots is influenced by both the number of nodes and the number of roots in each node (Hochholdinger, 2009). The node position is especially important because the nodal root whorls appear about 1 week apart (Picard et al., 1985) so over time more axial roots emerge and compete for the carbon being allocated to the root system, thereby influencing soil resource acquisition. The optimum number of roots is hypothesized to be greater in a low phosphorus soil relative to a low nitrogen soil (Lynch, 2013) because phosphate is relatively immobile in soil (Schenk and Barber, 1979) so more roots may increase the total phosphorus depletion volume, at least absent of interactions with plant metabolic economy.

The growth angle of nodal roots may be another important root phenotype that determines the overall shape of the root system and the distribution of roots in soil. Among maize varieties in the Wisconsin Diversity Panel (Hansey et al., 2011), the growth angle of crown roots ranged between 40° and 80° from horizontal across two seasons (Eric Nord, unpublished research). More shallow-angled primary axis root angles have previously been shown to decrease interplant competition for phosphorus between neighboring root systems of common bean by decreasing the extent of root overlap (Ge et al., 2000), and this logic may be even more important for a more mobile resource like nitrate where depletion zones caused by root uptake will tend to be larger and more likely to overlap. Intraplant competition will be decreased when nodal roots are more shallow-angled because the proximity of roots of the same plant will also be increased, as shown with basal root growth angle in common bean (Ge et al., 2000; Rubio et al., 2001). The optimal root phenotype
will likely depend on the agronomic conditions. Open-pollinated varietal mixtures of maize planted in mounds at very low population densities were common for thousands of years, often intercropped with common bean (*Phaesolous vulgaris*) and/or squash (*Cucurbita pepo*) (Hart, 2008). The advent of hybrid seed encouraged genetic monocultures and farm mechanization necessitated spatial arrangements convenient for planting and harvesting during the 1900s (Bogue, 1983). Over the past century, research on the role of plant nutrients encouraged farmers to amend soil with chemical forms of nitrogen and phosphorus (Russel and Williams, 1977). For more than 100 years, scientists have known that more productive land could accommodate a higher population density to optimize yield (Hume *et al.*, 1908). Over the past 70 years, greater fertilization of agricultural fields has supported greater population densities and increased yield (Dungan *et al.*, 1958). Maize population density increased from less than 30,000 plants ha\(^{-1}\) in 1930 to 80,000 plants ha\(^{-1}\) in 2005 (Duvick, 2005), and continues to increase in the USA. The agronomic conditions of global maize production vary greatly in availability of N and P in the soil due to fertilization and natural fertility, as well as in population densities. However, little is known about how nodal root number and growth angle function in different agronomic contexts, and how they interact to generate an integrated phenotype.

Early in the growth season, availability of nitrogen and phosphorus is greater in the topsoil (Jobbágy and Jackson, 2001) so relatively more shallow-angled roots that explore the topsoil facilitate resource acquisition during this stage (Lynch and Brown, 2001). However, nitrate leaches in agricultural systems so deep roots may be advantageous later in the season (Di and Cameron, 2002), even though most leaching occurs during fallow periods. In maize, more steep nodal roots were correlated with a deeper D\(_{95}\), the depth above which 95% of the root are located (Trachsel *et al.*, 2013), which could be beneficial for deeper soil resources like leaching nitrogen or water during drought. Due to these complexities, numerous knowledge gaps exist for how different numbers and growth angles of nodal roots may influence nitrogen and phosphorus acquisition.

In order to address the knowledge gaps associated with the optimal maize root system for different agronomic contexts, we simulated maize root phenotypes in contrasting nitrogen, phosphorus, and population density scenarios using *SimRoot*, a structural-functional model of root growth and architecture (Lynch et al. 1997; Postma and Lynch 2011). We hypothesized 1) fewer nodal roots will be beneficial for N uptake when N is limiting, 2) an intermediate number of nodal roots will be beneficial for P uptake when P is limiting, 3) fewer nodal roots will be beneficial at high population density, 4) steep nodal root growth angles will be beneficial for N uptake, 5) shallow nodal root growth angles will be beneficial for P uptake, 6) for both N and P limited
simulations, more shallow angles will be beneficial at high population densities and, 7) NRN and NRGA will have additive interactions.

**Methods**

In order to investigate the utility of nodal root number for soil resource acquisition and its interaction with nodal root angle in different environments, the functional-structural plant model SimRoot was used (Lynch et al. 1997; Postma and Lynch 2011). Essentially, SimRoot is a dynamic growth model of a 3-dimensional root system in virtual soil. For detailed information on the structure and function of SimRoot, readers are referred to Postma and Lynch (2011a) and (2011b), but the most pertinent details will follow. SimRoot simulations begin with both a starting seed and soil conditions. The seed produces root axes which grow and develop according to resource availability according to model parameters derived from empirical data. In this study, all plant properties remained the same in all simulations except for root architectural parameters as described below. The model includes a non-spatially explicit shoot model with canopy gas exchange and growth rates determined by resource availability and empirical parameters. Leaf expansion and photosynthesis are slowed by nitrogen and phosphorus stresses, and nitrogen and phosphorus stress also increase the relative carbon allocation to the root system. The soil model SWMS_3D (Simunek *et al.*, 1995) is used to simulate water and solute movement in the soil in conjunction with SimRoot, such that root uptake will result in loss of water, nitrate, and phosphorus from the soil which will drive water and nutrient flux within the soil.

Nodal root number is a phene aggregate that is determined by both the number of nodes and the number of roots in each node (Figure 1). Therefore, in order to understand the utility of this phene aggregate, the number of roots in each node must be varied in relation to the node position. In general, nodal root number increases over time because nodes of roots emerge successively as the maize root system develops. We chose to vary the number of nodes and nodal occupancy first by assuming that root systems primarily reduce nodal root number through a reduction of the number of nodes without many changes in the number of roots in each node. However, since the number of roots in a node may be independent of the number of nodes, we used the temporal aspect of nodal root emergence to generate phenotypes that produced the same number of nodal roots but produced them at normal, earlier, or later times. This temporal arrangement allowed the most logical assessment of phene state combinations. Table 1 summarizes the number of nodes and the
number of roots in each node for the three emergence time groups, and gives the emergence day of each node. *SimRoot* is parameterized for 40 days of growth which allows emergence of 5 nodes of crown roots and 1 node of brace roots.

The optimum nodal root number may depend on the identity of the soil resource, so all phenotypes contrasting for nodal root number and node emergence time were simulated at 5 soil availabilities of both nitrogen (N) and phosphorus (P) representing a continuum from severely stressed to non-limiting conditions, as defined by the maximum potential growth observed in the field. The soil parameters used in this study is a silt-loam common for agriculture soils with moderate nitrate leaching potential and with the majority of P found in the upper 20 cm. In order to understand the utility of nodal root number in different phenotypic and environmental contexts, the full set of nodal root number phenotypes with normal emergence time were simulated in factorial combination with 8 levels of nodal root angle ranging from 10 to 80 degrees from horizontal. All these phenotypic combinations were simulated at 40,000, 80,000, and 120,000 plants ha⁻¹ at moderately high and low nitrogen availability, 100 and 40 kg N ha⁻¹, and at moderately high and low phosphorus availability, 30 and 12 kg P ha⁻¹. These conditions represent a continuum between the agronomic contexts of subsistence to commercial agriculture.

**Results**

Under the most severe N and P limitations, low N (LN) and low P (LP), the effects of nodal root number and emergence time on shoot mass were fairly similar (Figs. 2, 3). Plants with fewer nodal roots had the greatest shoot mass, with the greatest shoot mass at zero nodal roots. Regardless of nodal root number, early emerging systems performed the worst and late emerging systems performed the best. At intermediate availabilities of N and P (MN and MP), the effects of nodal root number and emergence time on shoot biomass were somewhat different from each other and from the lower availability simulations. In MN, the phenotype with zero nodal roots did not have the greatest shoot mass, rather greatest shoot mass was reached by an intermediate, but still low, number of nodal roots. In MN, phenotypes with later emerging nodal roots had the greatest shoot mass, but the optimum NRN shifter to a greater number relative to LN. In MP, phenotypes with an intermediate number of nodal roots had the greatest shoot mass, however the phenotypes with normal emergence timing performed better than late and early emerging phenotypes. Under low N and P availabilities, total root length had a similar response as shoot mass in LN and LP, with
phenotypes with fewer nodal roots emerging later having the greatest total root length (Fig. 5). However, in MN, the total root length was greatest at intermediate numbers of nodal roots that emerge early (Fig. 6). In MP, total root length increased with more nodal roots that emerged early (Fig. 6).

In the simulations in moderately high availability of nitrogen, population density had a relatively small effect on shoot mass (Fig. 8). Shoot mass decreased from an average of 26 g at the lowest density to an average of 22 g at the highest density. Population density affected the optimal NRN and NRA combination. At the lowest density (40k), there was no difference in shoot mass among the different NRN x NRA combinations, demonstrating that in this scenario nutrients were non-limiting. At the medium density (80k), systems with fewer nodal roots generally had greater shoot mass, and intermediate angles performed better at lower NRN, but at greater NRN more shallow angles performed best. The shoot mass gain at the highest density was even greater for few NRN and intermediate angles performed best over a range of NRN from 10 to 30.

In the simulations at moderately low availability of nitrogen, planting density had a more pronounced effect on shoot mass than in moderately high N, with a 50% decline from 40k to 120k (Fig. 9). In general, phenotypes with fewer nodal roots performed better than those with many regardless of phenotypic and agronomic context. At 40k, most angles performed similarly, except the most steep (80º) performed worse. At 80k, phenotypes with intermediate to shallow angles had the greatest shoot mass. In the highest density, the most shallow phenotype had the greatest shoot mass across a wide range of NRN from 10 to 40.

In the simulations in moderately high availability of phosphorus, planting density had little effect on shoot dry weight (Fig. 10). In general, an optimum NRN was reached around 20 nodal roots for all angles and planting densities, and the most shallow-angled phenotypes had the greatest shoot mass. Results for the moderately low phosphorus level were similar to results at the moderately high P level except for a general decrease in shoot dry weight (Fig. 11).

**Discussion**

This study investigated the relationships of nodal root number, position, nodal occupancy, and nodal root growth angle with N and P acquisition and plant growth *in silico*. At the lowest availabilities of N and P, root phenotypes with no nodal roots and only a seminal root system had the greatest shoot growth, while at intermediate availabilities of N and P an optimum NRN occurred.
with relatively few nodal roots. In general, delayed emergence of nodal roots was beneficial for plant growth. Greater planting density decreased shoot mass in the nitrogen simulations, but had no effect in the phosphorus simulations. In the nitrogen simulations, phenotypes with zero or very few nodal roots performed best and generally intermediate angles had greater shoot mass, though at the highest density with moderately low availability of nitrogen, the most shallow phenotype had the greatest shoot mass over a wide range of NRN. In both moderately high and low levels of phosphorus, a strong optimum for NRN existed around 15 nodal roots, and extremely shallow-angled systems performed better regardless of NRN.

Recently, experiments using recombinant inbred lines (RILs) of maize in the field and greenhouse by Saengwilai et al. (2014) demonstrated that root systems with fewer crown roots maximized N acquisition and yield in LN soil and had greater D95, the depth above which 95% of root length is located. Another field study using 100 maize RILs observed a negative correlation of nodal root number with grain yield, especially in LN soil (Gallais and Coque, 2005). In our simulations, the loss of nodal roots as a resource sink allowed lateral roots to become longer. A similar phenomenon was described for maize in aeroponics where root systems grown with low nitrogen solution had 36% more lateral root length per length of crown root (Gaudin et al., 2011), which validates this model response. An important contribution to maize breeding could possibly be made if a low crown root number, long lateral root integrated phenotype could be an intrinsic architecture rather than a plastic response to nitrogen stress. A study of root system variation among maize landraces in the field demonstrated greater P-efficiency associated with greater numbers of nodal roots (Bayuelo-Jiménez et al., 2011). The fewest crown roots (not counting brace roots) observed in these field studies was 30, and the results from the current simulations suggest even fewer may be desirable to optimize both N and P acquisition, especially in conditions of higher stress such as extremely low fertility and high population densities. Reduction of NRN within emergence time groups by eliminating later emerging nodes is justified by earlier work with the rootless maize mutant which had 40% as many nodal roots as the normal type and did not form roots on the last three whorls observed in the normal type (Jenkins, 1930).

The mechanisms giving rise to nodal root growth angle of maize and Arabidopsis, or the related phenomenon of plagiogravitropism, have been relatively well-studied (Jackson and Barlow, 1981; Nakamoto, 1994; Band et al., 2012), however there have been few direct links between NRGA and functional utility for the acquisition of soil resources. The relative intensity of foraging at different depths, such as shallow versus deep foraging, has been assumed to be an outcome of NRGA. For example, a field study maize showed topsoil foraging as determined from soil cores
was important for acquisition of phosphorus, but nodal root growth angle was not measured (Zhu et al., 2005). Similarly, maize lines with deeper rooting had greater shoot mass and transpiration in greenhouse mesocosms simulating drought (Hund et al., 2008). Steep nodal root growth angles were correlated with deeper D95 in the field (Trachsel et al., 2013). In rice, the Dro1 QTL was discovered by using an index for nodal root growth angle and plants with steeper roots were found to also have deeper roots in the field (Uga et al., 2011). However, rooting depth is also influenced by other phene aggregates such as crown root number (Saengwilai et al., 2014b), so future studies must distinguish the component phenes of aggregates such as rooting depth (York et al., 2013). In common bean, direct associations have been made between basal root growth angle that is shallow and increased P acquisition efficiency in the field (Bonser et al., 1996; Liao et al., 2004), and between shallow angle and decreased interplant and intraplant competition in the field (Rubio et al., 2003) and in simulation studies (Ge et al., 2000; Rubio et al., 2001). Our simulations for nodal root growth angle in maize agree with the general conclusion that shallow growth angles of axial roots benefit P acquisition for crops, and that intermediate to shallow angles benefit N acquisition, especially in the case of competition such as that caused by high population density.

Little is known about how maize lines differ in the timing of the emergence of nodal roots from specific shoot nodes. When details are given about the timing of node emergence, only one line may be reported (e.g. Picard et al. 1985), or else an incomplete measurement of only a few nodes is conducted for many lines (e.g Guingo et al. 1998). The relationship between fewer nodal roots and longer lateral roots observed in our simulations accentuates a phenotyping challenge where more detail is needed about nodal root system patterns and other aspects of nodal root phenotypes like lateral root branching and length. One approach to increase throughput of field phenotyping is the use of digital images of excavated root crowns combined with automatic image analysis (Grift et al., 2011; Bucksch et al., 2014), however the use of mature root systems prevents measurements of the occluded part of the root system, and neither of these image analysis platforms count individual nodal roots. However, imaging of mature root crowns can be conducted while excising whorls of nodal roots to reveal interior whorls, and nodal roots can either be counted manually or automatic counting may be feasible by software. Phenotyping of mature root systems does not permit analysis of the temporal aspects of nodal root emergence. The use of mesocosms containing clear gels has been another approach that allows detailed architectural analysis over time, however growth is limited to young plants and such media may introduce artifacts due to its dissimilarity to field soil (Iyer-Pascuzzi et al., 2010). Micro-CT using X-rays is another promising approach to visualize roots over time in soil, however pot size currently limits growth to young
plants (Mooney et al., 2011). Likewise, measurements of nodal root growth angles in the field have been limited to the outer brace and crown roots (Trachsel et al., 2011), and measurements of angle in containers may not be valid because of the physical constraints of container size. In the near future, weekly field measurements, manually counting nodes and the number of roots in each node may, along with measuring NRGA for each node, be the only way to adequately capture the spatiotemporal dynamics of the maize nodal root system.

Nitrate leaching in an important aspect of agricultural systems that will influence the vertical distribution of nitrate in soil. In some systems, a great amount of nitrogen may be immobilized by microorganisms, but as that nitrogen is mineralized then it may leach, especially with high rainfall. Most nitrate leaching below the root zone occurs during the fallow periods between cropping seasons (Di and Cameron, 2002) but some may leach to greater depths during the season (Thorup-Kristensen et al., 2009). Deep rooting will be especially important when nitrate leaching is greater. In the current simulations, leaching was moderate, and partially offset by mineralization in the shallow layers.

In light of these results, the root phenotypes of many elite maize lines may have more nodal roots than what we might predict to be optimal for nutrient acquisition. Possible explanations for this include: genetic selection in high fertility soils, fitness tradeoffs resulting from less nodal roots, and genetic constraints in nodal root production. Breeding for high-yield maize typically occurs under optimum N and P fertility (Lafitte and Edmeades, 1994). Our results and those from a previous field study (Saengwilai et al., 2014b) demonstrate that maize shoot mass is insensitive to variation in nodal root number when soil resources are adequate. When nutrients are non-limiting, cost-saving phene states like decreased nodal root number may be masked. Tradeoffs may also exist for fewer nodal roots, even if there were benefits for nutrient acquisition. One such tradeoff is an increased risk of crop failure due to root herbivory and diseases (Whitfield, 1992). Plants may over-proliferate roots in order to mitigate the chance that too many roots could be lost to maintain adequate shoot growth. Another tradeoff may be an association of fewer nodal roots, especially brace roots, with lodging, although this association is neither strong (Varlet-Grancher et al., 1987) nor consistent (Stamp and Kiel, 1992). Last, all grasses produce layers of nodal roots (Singh et al., 2010), and maize may be constrained by its genetics and development to produce what is above-optimum numbers of nodal roots with regards to nutrient acquisition. Similarly, the genetics of maize could be constrained by the few founder lines responsible for many commercial maize hybrids (Smith et al., 2004). Nodal root growth angle is commonly greater than 45º from horizontal, and as steep as 80º (Eric Nord, unpublished research; York, unpublished research), although work
from the early 1900s depicts very shallow-angled root phenotypes of maize (Weaver, 1926; Kiesselbach and Weihing, 1935). Identification of genetic sources conferring decreased nodal root number and more shallow nodal roots could allow introgression of novel and potentially useful variation for root phene states into elite maize lines.

Acquiring resources before a competitor may increase relative fitness and answer the question of ‘why plants bother’ to proliferate roots (Hodge et al., 1999). In another simulation study, phenes and phene aggregates affecting the intensity of soil exploitation, such as greater lateral branching, ranked highly among many root and soil properties for their influence on crop-weed competition (Dunbabin, 2007). Increasing fitness relative to competitors is important in natural systems, but can lead to a ‘tragedy of the commons,’ which is a common prediction of game theory with regards to community dynamics (Hardin, 1968). In the case of roots, game theory predicted a plastic response that over-proliferated roots relative to the optimal amount of roots to maximize uptake efficiency of a single plant, in order to preempt a competitor from acquiring soil resources (Gersani et al., 2001). However, evidence for the ‘tragedy of the commons’ as a plasticity response with regards to roots is conflicting (Semchenko et al., 2007; Dudley and File, 2007; Nord et al., 2011). In contrast, avoidance of root growth redundancy, or over-proliferation, has been hypothesized to be important for agriculture systems where optimizing yield of the focal crop is the goal (Zhang, 1999). Therefore, the optimal NRN may be greater in natural systems than in agricultural systems, and earlier production of nodal roots may also maximize relative fitness. NRGA would be expected to maximize relative fitness in natural systems but maximize acquisition efficiency in agricultural systems.

Simulation modeling provides an opportunity to explore the differences among more phenotypes in more environmental contexts than is possible through empirical experimentation, and also in greater detail, in such a way as to provide a positive feedback loop between theoretic and empirical research (Wullschleger et al., 1994). Aspects of the current simulation are validated by previous work, such as the improved N acquisition and deeper rooting of systems with fewer nodal roots (Saengwilai et al., 2014b), and the increased lateral root length of systems with fewer nodal roots (Gaudin et al., 2011). SimRoot was not parameterized based on these studies but rather relies on more fundamental resource dynamics (Postma and Lynch 2011), so the fact that our results agree with empirical results is a validation of the model results. These simulations have demonstrated that the increased lateral root length conferred by fewer nodal roots benefits N acquisition, similar to another study that intentionally varied frequency of lateral root branching and demonstrated fewer but longer laterals were beneficial for N acquisition (Postma et al., 2014).
The current simulations allowed the generation of novel maize phenotypes not observed in the field, such as with zero nodal roots and initial nodal root growth angles that are almost horizontal, and demonstrated these extreme phenotypes could affect N and P acquisition. The timing of nodal root emergence, or the balance of the number of nodal roots in different node positions, is very difficult to study in the field, but these simulations demonstrated that later emerging nodal roots confer a growth advantage. Simulation modeling can also be used to address the knowledge gaps associated with the functional integration of root phenes (York et al., 2013), such as nodal root number and nodal root growth angle. NRN affects carbon economy directly, whereas NRGA does not, so the beneficial states of these phene aggregates have no tradeoff and produce additive effects on shoot growth. Most field studies of the functional utility of maize root phenes and aggregates occur at a single density, yet we have demonstrated an influence of density on the optimum root system phenotype for N acquisition. Future studies should identify a target agronomic context that is most relevant for the phene or aggregate in question in order to maximize the potential impact of the study. We have shown that more detailed analysis of the spatiotemporal dynamics of the maize root system, such as the number of roots in all nodes and their growth angles, has the potential to provide benefits for maize breeding programs.

**Acknowledgements**

We thank Jouke A. Posma, the creator of the current version of *SimRoot*, for advice on using the model, and thank Harini Rangarajan for providing a program converting *SimRoot* VTU visualization files to text files for data analysis. This work was supported by Agriculture and Food Research Initiative competitive grant number: 2014-67013-2157 of the USDA National Institute of Food and Agriculture to JPL.

**References**


Figure 5-1. Two simulated maize root systems grown without stress simulation until 40 days of age are shown with seminal roots in red and nodal roots in blue. The root system on the left has 6 nodal roots and the system on the right has 60.
Figure 5-2. The 40 day old root system of a great NRN simulation is shown on the left, while a small NRN simulation is shown on the right. Both simulations occurred in a low nitrogen soil (40 kg N ha$^{-1}$) and have moderate nodal root angles. Fewer nodal roots allow more allocation to lateral roots and positive feedbacks with shoot growth allow greater allocation of carbon to the root system. Mirroring of the roots is caused by the simulation of planting density, so that the roots in the soil more closely mimic field conditions.
Figure 5-3. Shoot dry weight for simulated maize plants at 40 days of age is shown for plants grown in LN (A) and LP (B) as determined by nodal root number and timing of nodal root emergence.
Figure 5-4. Shoot dry weight for simulated maize plants at 40 days of age is shown for plants grown in MN (A) and MP (B) as determined by nodal root number and timing of nodal root emergence.
Figure 5-5. Total root length is for simulated maize plants at 40 days of age shown for plants grown in LN (A) and LP (B) as determined by nodal root number and timing of nodal root emergence.
Figure 5-6. Total root length for simulated maize plants at 40 days of age is shown for plants grown in MN (A) and MP (B) as determined by nodal root number and timing of nodal root emergence.
Figure 5-7. Maize plants were simulated at 40,000 plants ha$^{-1}$ (left), 80,000 plants ha$^{-1}$ (middle), and 120,000 plants ha$^{-1}$ (right). This example simulation is from a nodal root number of 22 and a nodal root angle of 20 degrees from horizontal, near the optimum for many simulations following, at the moderately low level of nitrogen (40 kg N ha$^{-1}$). Simulation views across the top are from above, while those across the bottom are from the side. The distance between rows remained constant at 76 cm in all simulations and the distance between plants was varied to generate the desired densities.
Figure 5-8. Shoot dry weight at a moderately high nitrogen level (100 kg N ha$^{-1}$) is shown as determined by nodal root number, nodal root angle, and density in simulations of maize root systems at 40 days of age. Densities are 40,000, 80,000, and 120,000 plants ha$^{-1}$. Simulations of different angles are shown as different colored lines where angle is expressed as degrees from horizontal in the legend. Lines are smoothed by Loess.
Figure 5-9. Shoot dry weight at a moderately low nitrogen level (40 kg N ha⁻¹) is shown as determined by nodal root number, nodal root angle, and density in simulations of maize root systems at 40 days of age. Densities are 40,000, 80,000, and 120,000 plants ha⁻¹. Simulations of different angles are shown as different colored lines where angle is expressed as degrees from horizontal in the legend. Lines are smoothed by Loess.
Figure 5-10. Shoot dry weight at a moderately high phosphorus level (40 kg P ha$^{-1}$) is shown as determined by nodal root number, nodal root angle, and density in simulations of maize root systems at 40 days of age. Densities are 40,000, 80,000, and 120,000 plants ha$^{-1}$. Simulations of different angles are shown as different colored lines where angle is expressed as degrees from horizontal in the legend. Lines are smoothed by Loess.
Figure 5-11. Shoot dry weight at a moderately low phosphorus level (15 kg P ha\(^{-1}\)) is shown as determined by nodal root number, nodal root angle, and density in simulations of maize root systems at 40 days of age. Densities are 40,000, 80,000, and 120,000 plants ha\(^{-1}\). Simulations of different angles are shown as different colored lines where angle is expressed as degrees from horizontal in the legend. Lines are smoothed by Loess.
Table 5-1. Contributions of the node positions and number of roots in each node are shown for determining the total nodal root number for 3 timing models of early emerging, normal emerging, and late emerging nodal roots. Crown root nodes (CR1-5) and the brace root node (BR1) emerge on days listed at the top, which are the same for early, normal, and late emerging models. The nodal root number (NRN) column sums the number of roots occupying all root nodes for each row. Normal time of emergence represents the emergence times commonly used in SimRoot as measured in greenhouse experiments, while the early time places a higher proportion on the earlier developing nodes, and the late time places a higher proportion of roots on the later forming nodes.

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Chapter 6 Evolution of US maize (*Zea mays* L.) root system architectural and anatomical phenes over the past 100 years corresponds to increased tolerance of nitrogen stress

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Abstract

Increasing the nitrogen use efficiency of maize is an important goal for food security and agricultural sustainability. In the past 100 years, maize breeding has focused on yield and aboveground phenes. Over this period maize cultivation has changed from low fertilizer inputs and low population densities to intensive fertilization and dense populations. We hypothesized that through indirect selection the maize root system has evolved phenotypes suited to more intense competition for N. Sixteen maize varieties representing commercially successful lines over the past century were planted at 2 nitrogen levels and 3 planting densities. Root systems of the most recent material were 7º more shallow, had 1 less nodal root per whorl, had double the distance from nodal root emergence to lateral branching, and had 14% more metaxylem vessels, but total mextaxylem vessel area remained unchanged because individual metaxylem vessels had 12% less area. We also observed plasticity in cortical phenes such as aerenchyma, which increased at greater population densities. Simulation modeling with *SimRoot* demonstrated that even these relatively small changes in root architecture and anatomy could increase maize shoot growth by 16% in a high density and high nitrogen environment. We conclude that evolution of the maize root system over the past 100 years is consistent with increasing nitrogen use efficiency. Introgression of more contrasting root phene states into the germplasm of elite maize and determination of the functional utility of these phene states in multiple agronomic conditions could contribute to future yield gains.
Introduction

Global food production must double in order to meet the demands of the future population of 9 billion people predicted by 2050 (Royal Society, 2009). There is a shortage of arable land (Pretty, 2008), so land use efficiency must increase dramatically to meet current and future demand. Maximizing crop nutrient and water use efficiencies is one approach for increasing land productivity (Lynch, 1998). Average maize production per hectare in the USA has increased about 8-fold in the past century (USDA-NASS, 2013). Genetic improvement and agronomic practices have contributed to this increased production about equally (Duvick, 2005). Increased yield in maize due to breeding has been associated with changes in a variety of phenes (i.e., basic units of phenotype, sensu Serebrovsky, 1925 and Lynch, 2011, see York et al. 2013 for discussion), including phenology, leaf angle, and kernel number, among others (Tollenaar and Lee, 2006). Newer maize hybrids may also have increased tolerance to soil resource constraints such as low soil nitrogen (N) (McCullough et al., 1994a) and drought (Dwyer et al., 1992).

Over the past 100 years, maize breeding has continually adapted to changing agronomic conditions. Maize crop stands existed as widely-spaced, open-pollinated varietal mixtures for thousands of years until the advent of hybrid seed encouraged genetic monocultures and the mechanization of farm tools necessitated spatial arrangements convenient for planting and harvesting between 1910 and 1940 (Bogue, 1983). During the same period, research on the role of plant nutrients encouraged farmers to amend soil with more nitrogen in organic and chemical forms (Russel and Williams, 1977). Subsequently, world chemical nitrogen use increased 3-fold between 1930 and the end of WWII, due mostly to application over a greater area (Frink et al., 1999). Since 1960 the use of chemical nitrogen fertilizer increased substantially in the USA from an average of 65 kg ha⁻¹ to 160 kg ha⁻¹, an increase of 240% (USDA-ERS). In the early 1900s researchers were already aware that more productive land could accommodate a higher population density to optimize yield (Hume et al., 1908), and by mid-century the connection was made that nitrogen fertilization could support higher population densities to increase yield (Dungan et al., 1958). Maize population density increased from less than 30,000 plants ha⁻¹ in 1930, to 40,000 plants ha⁻¹ in 1960, and then doubling to an average of 80,000 plants ha⁻¹ in 2005 (Duvick, 2005), and continues to increase. Maize in the USA has been selected to maximize yield in these changing intensive management systems, and evolution of the root system is hypothesized to have occurred to increase resource acquisition efficiency in these systems.
Maize performance is influenced by many root system architectural, anatomical, and morphological phenes and phene aggregates (see Lynch 2014, Lynch and Brown, 2012; York et al., 2013) that influence root distribution and soil resource acquisition, including crown root number (York et al., 2013; Saengwilai et al., 2014b) topsoil foraging (Zhu et al., 2005a), crown root angle (Trachsel et al., 2013), rooting depth (Hund et al., 2008), lateral branching (Zhu and Lynch, 2004; Zhu et al., 2005b; Postma et al., 2014), root cortical aerenchyma (RCA) (Zhu et al., 2010a; Saengwilai et al., 2014a), living cortical area (Jaramillo et al., 2013), cortical cell size (Chimungu et al., 2014a), cortical cell file number (Chimungu et al., 2014b), and root hairs (Zhu et al., 2010b). Root phenes may also influence interplant competition and facilitation (Hodge et al., 1999; Ge et al., 2000; Bates and Lynch, 2001; Rubio et al., 2003; Postma and Lynch, 2012; Zhang et al., 2014). Interactions among root phenes for foraging utility and metabolism economics will determine how these root phenes influence soil source acquisition together to create a functionally integrated phenotype (York et al., 2013). Simulation studies suggested that deeper roots which access more stored water may drive better performance of modern hybrids at higher plant densities (Hammer et al., 2009). An ideotype of maize root phenotypes for water and nitrogen acquisition has been proposed consisting of several interacting architectural, anatomical, and physiological phenes (i.e., steep, deep, and cheap, or ‘SCD’; Lynch 2013). However, breeding in maize over the past 100 years has been primarily driven by selection for aboveground phenes and yield, with less understanding of root phene utilities and whether the root system has evolved over this period.

We hypothesize that breeders indirectly selected the maize root system over the past 100 years through the root system’s influence on yield. Specifically, we propose maize root architectural and anatomical phenes have evolved towards the states represented by SCD ideotype (Lynch, 2013), which will be used here as a reference phenotype. We also hypothesize these root phenes exhibit plastic responses to varying nitrogen and density levels representing changes in US agronomic practices, but that plasticity will not disrupt the overall pattern of more contemporary maize cultivars exhibiting more SCD-like phenotypes. In order to test these hypotheses, 16 maize varieties spanning the past century were grown in two nitrogen levels and three densities.
Methods

Plant Material

One open-pollinated variety (OPV) and 15 hybrids (Table 1) were evaluated as representatives from the entire Dupont Pioneer Era panel (Duvick et al., 2004) which contains well-studied, commercially successful material from the past century in the USA. The release dates of the present material range from 1900 until 2011.

Experimental Site

The experiment was conducted in 4 fields at the Russell Larson Research Farm (aka Rocksprings) of the Pennsylvania State University (40°42'40.7" N 77°57'11.1" W). The soil was a Hagerstown silt loam (fine, mixed, semiactive, mesic Typic Hapludalf).

Treatment Installation

The experiment included 4 blocks with each block being a separate 0.4 hectare field. Each field was split in half randomly. On one half of each field, no nitrogen fertilizer had been applied since 2010, while on the other half 145 kg ha⁻¹ of nitrogen was applied each year for the previous 2 years. In May 2013, entire fields received phosphorus (P) and potassium as determined by soil tests. On one half of each field, 145 kg ha⁻¹ elemental N as dry urea granules was broadcast to produce a high nitrogen (HN) plot, while the low nitrogen (LN) side received no N fertilizer. Following, entire fields were disked to incorporate the fertilizers. A planter passed through all fields leaving behind non-planted rows in which to plant manually. Within these HN and LN split-plots, the factorial combinations of the three densities and 16 varieties were completely randomized. The three densities were 20,000, 40,000, and 80,000 plants per hectare, which are abbreviated as 20K, 40K, and 80K, throughout the manuscript and in figures. Each nitrogen level, density level, and specific variety combination was planted in a 5 row plot that was 5 meters long with a row distance of 76 cm. Seeds were hand-planted into rows marked by the planter using stakes and ropes marked to accommodate the various densities on June 1, 2014. Irrigation was not required because of
adequate rainfall, insecticides were not required, and herbicide was applied before and after planting.

**Experimental Sampling and Harvest**

Shoots and root crowns were sampled from one block each day between August 12-15, 2013 (70 DAP). Three entire plants were excavated and processed in the field from each plot using the shovelomics method (Trachsel et al., 2011) with the shovel inserted 30 cm from the base of the plants. Shoots of the three plants were combined and partitioned into leaves, stalks, and immature ears, if any. The three root crowns were soaked in soapy water to disperse clay then washed with a water hose and nozzle until most soil was removed. One representative root crown was selected for subsequent architectural measurements from the three root crowns that had been excavated based on its apparent average size and uniformity, and a second root crown was selected for anatomical sampling. All shoots were dried at 60º C for two weeks and dry mass for leaves, stalks, and ears was measured. Leaves were ground in a Wiley mill with a 40-mesh sieve (Thomas Scientific, Waltham, MA, USA) and a subsample analyzed for nitrogen content with an elemental analyzer (PerkinElmer 2400 Series II, Swedesboro, NJ, USA). Grain yield samples were collected from 5 random plants in each plot on October 13, 2013, threshed, and weighed.

**Root Crown Imaging and Architectural Measurement**

The original shovelomics method (Trachsel et al., 2011) was modified to accelerate field processing while permitting more intensive measurements. Root crowns were kept in large plastic bins submerged in water inside a 5º C cold room until they were imaged within one week. Root crowns were imaged using digital cameras attached to frames with camera mounts such that the camera was facing down from a height of 50 cm. Three identical cameras (Canon PowerShot A1200) operated by three researchers were used to process samples quickly. Root crowns were placed under the camera on a matte black background. A 3 cm white plastic disk was included as a scale in every image, along with a printed sample label. Camera zoom and focus were kept locked for the duration of the imaging. An image was taken of the outermost layer of brace roots (aboveground nodal roots) that penetrated the ground, and of the outermost layer of crown roots.
(belowground nodal roots), which is the last whorl of nodal roots that forms belowground during development. The whorl of crown roots was revealed by excising one whorl of brace roots at a time in order to reveal the outer crown roots. A representative nodal root of the respective whorl was excised from the side of the root crown not facing the camera and placed to the side of the root crown such that both root crown and representative root were in frame of the image.

Image analysis was conducted in RSAJ² which is a project for the ObjectJ plugin (Vischer and Nastase, 2009) for ImageJ (Schneider et al., 2012). RSAJ prompts the user to take sequential measurements from the images (Fig. 1), which are briefly described (see RSAJ manual for elaboration). The same measurements were taken for the outermost brace roots and the outermost crown roots. Nodal root angle from horizontal was derived trigonometrically from the stem width, maximum root crown width, and the height between stem width and root crown width. The number of nodal roots counted for a whorl was multiplied by 2 in order to account for the occluded half of the root system, based on previous observations. The diameter of the representative nodal root was measured at its base, along with the distance from where the representative root was excised from the shoot to where lateral roots emerge (distance to branching), which is equivalent to the distance from the stem to the first lateral. In order to calculate lateral root branching density, the number of lateral roots was counted along a measured length on the representative nodal root. Finally, the lengths of three representative lateral roots were measured and averaged for analysis. See Table 2 for a list and explanation of all measured root phenes.

Anatomical Sampling, Imaging, and Measurement

In the field, one washed root crown per plot was processed for collecting anatomical samples. The middle part of a root from the second or third node that develops is the most representative for anatomical studies of maize roots (Burton et al., 2013). Consequently, two nodal roots from the second whorl, which is found in the center of the root crown, were excised and a 4 cm segment was extracted 8 cm from the origin of the focal root from the stem and preserved in 75% ethanol until samples could be processed. One root segment per sample was processed using laser ablation tomography. Briefly, a sample was mounted on a stage moving at a rate of 30 μm s⁻¹ perpendicular to a laser beam, with the length of the root being in the same direction as stage

¹ Available at: http://plantscience.psu.edu/roots/methods/computer/RSAJ
movement. The root cross-sectional surface was ablated as it traveled through the 355 nm ultraviolet laser beam pulsed at 37 kHz (AVIA 355-7000, Coherent, Santa Clara, CA, USA). The exposed cross-sectional surface was imaged with a Canon EOS REBEL T3i camera using a 65mm f/2 8-16 lens. The three most clear and representative images for each segment were analyzed with the semi-automated root anatomical measurement software RootScan (Burton et al., 2012). Briefly and non-exhaustively, RootScan automatically separates the cortex from the stele, and within the cortex measures the total area of cortex, the average size of cortical cells, and the area of aerenchyma, while within the stele the number and areas of xylem vessels are measured. See Table 2 for a list and explanation of all reported architectural and anatomical root phenes.

Simulation Modeling

In order to investigate the relationship between changes in root system architecture over time and the functional utility for N acquisition, root systems representing the oldest and newest germplasm measured in this study were modeled in SimRoot (Lynch et al. 1997; Postma and Lynch 2011). For detailed information on the structure and function of SimRoot and its default parameters, readers are referred to Postma and Lynch (2011a) and (2011b), but the most pertinent details will follow. SimRoot simulations include both a starting seed and soil conditions, where the soil is defined by soil, water, and nitrate properties. At the starting time, the seed produces root axes based on growth of real plants and with properties defined by the input files. In this study, all plant properties remained the same among simulations except for architectural and anatomical parameters as described below. The model includes a non-spatially explicit shoot model with expansion of leaf area leading to increased photosynthesis, and with growth rates constrained by maximums measured in real plants. Maximum growth rate is decreased proportionally to increasing nitrate stress, and nitrate stress will also increase the relative carbon allocation to the root system. The soil model SWMS_3D (Simunek et al., 1995) is used to simulate water and solute movement in the soil, such that root uptake will result in loss of water and nitrate from the soil which will drive water and nitrate flux within the soil. The simulated soil used in the current study was modeled after the same soil in which the field experiment took place with similar rainfall. The simulation time was 40 days.

An Old and a Modern phenotype were simulated which were identical except the Modern phenotype was 10 degrees more shallow-angled and had 1 less nodal root in every whorl, which
captures the essence of architectural changes observed in this study (Fig. 2). Three variants of the Old phenotype were simulated: 1) typical (Old) 2) typical with more shallow angle (Old + Angle) and 3) typical with fewer nodal root number (Old + NRN). In this way, the contribution of each phene was assessed in isolation and combined. Two versions of the Modern phenotype were simulated: 1) typical (Modern) 2) typical with 40% RCA (Modern + RCA). Though RCA did not change among Era periods in this study, RCA might be an important phene for breeding programs so was simulated. These 5 phenotypes were simulated at a high density with high nitrogen availability (HDHN), and at a low density with low nitrogen availability (LDLN), similar to the extremes of the field study.

Statistical Analysis

In order to simplify data interpretation, the 16 varieties were grouped into 4 groups of 4 varieties (Era periods) based on their similarities of release year, original agronomic context, and breeding method (Table 1). All statistics were conducted and data graphics were created with R version 3.0.2 (R Core Team, 2013). Analysis of variance (ANOVA) was conducted with the mixed modeling package nlme (Pinheiro et al., 2013) in R with N level nested in block as the random effect and with Era period, density, and N level as fixed effects. Post-hoc mean comparisons were conducted between Era periods when effects from ANOVA were significant (p < 0.05) with Tukey HSD using the multcomp package (Torsten et al., 2008). Principal component analysis (PCA) was conducted on complete observations of crown root architectural and anatomical raw data (n = 261). Brace root architectural data were excluded for PCA because many brace roots did not form lateral roots which substantially reduced the number of complete observations. Graphs were created in R with the ggplot2 package (Wickham, 2009).

Results

Shoot Mass, Nitrogen Content, and Yield (Table 3)

Total biomass produced on a per area basis did not differ among Era periods (p = 0.6122), but plants grown under LN were 16% less massive than those under HN (p = 0.035), and there was
a 50.3% reduction in shoot mass per area from 80k to 20k (p < 0.0001). Percent nitrogen in the leaves was not affected by Era (Fig. 3A) but decreased 49.6% from HN to LN (p = 0.0003), and increased 29.4% from 80k to 20k (p < 0.0001). Grain yield on a per area basis (Fig. 3B) was significantly affected by all treatments, with a 52% reduction in yield from HN to LN (p = 0.0013), a 66% increase from 20k to 80k plants ha\(^{-1}\) (p < 0.0001), and a 58% increase in yield from the oldest to the newest lines (p < 0.0001).

**Root System Architecture (Tables 4, 5)**

Brace root angle (Fig. 4A) decreased 12% from 59º to 52º from horizontal (p < 0.0001) from the oldest to the newest Era period, indicating that varieties in the most recent Era period are relatively more shallow-angled than in the oldest. Brace root angle was more shallow-angled at lower density, decreasing 5% from 57º to 54º degrees from horizontal between 80k and 20k (p = 0.0171). Crown root angle (Fig. 4B) of the most recent material also decreased 10% from 62º to 55º from horizontal, compared to the older materials (p < 0.0001). A t-test demonstrates that crown roots are steeper than brace roots overall, with means of 58.4º and 55.6º from horizontal, respectively (p < 0.0001). Brace and crown root angles are greater than 45º from horizontal (p < 0.0001).

Independently, brace and crown root numbers were not significantly affected by Era period. However, the combined brace and crown root number (Fig. 5) decreased from 19.2 in the oldest period to 17.6 in the newest period (p = 0.0083). Combined root number decreased from 19.4 in HN to 17.4 in LN (p = 0.0197). High density decreased combined root number from 20.2 in low density to 16.3 (p < 0.0001).

Distance to branching of both brace roots and crown roots increased considerably from older material to newer material (Fig. 6). For brace roots (Fig. 6A), distance to branching increased 50% from 3.45 cm to 5.17 cm between the oldest Era period and the most recent (p = 0.0005). Distance to branching was decreased from 6.16 cm to 2.7 cm from 20k to 80k planting density (p < 0.0001), and decreased from 5.1 to 3.65 from HN to LN (p = 0.0126), in brace roots. For crown roots (Fig. 6B), distance to branching increased 57% from .73 cm to 1.15 cm between the oldest Era period and the most recent (p = 0.0019), but was not affected by density or nitrogen level.

Lateral root length was measured for 3 random lateral roots on each excised representative root and those three values averaged for subsequent analysis. For brace roots (Fig. 7A), lateral root
length was not affected by Era period or nitrogen level, but was affected by density \((p = .0117)\), decreasing from 3.6 cm to 2.7 cm from 20k to 80k (Fig. 6). However, the length of crown root lateral roots (Fig. 7B) was significantly affected by Era period, increasing 29% from 3.73 cm to 4.83 cm from the oldest to newest period \((p < 0.0001)\). Crown root lateral roots increased in length from 3.66 cm from 4.22 cm from 20k to 80k density \((p = 0.0325)\).

Brace and crown lateral root densities were not affected by Era period. Brace root lateral root density increased 15.8% from 8.42 laterals cm\(^{-1}\) to 9.75 laterals cm\(^{-1}\) from 20k to 80k \((p = 0.0445)\), but was not affected by nitrogen level. Crown root lateral root density was not affected by any treatment.

**Root Anatomy (Table 6)**

Percent cortical aerenchyma was not affected by Era period, but increased from 10.5% to 18.8% of cortical area (Fig. 8A) from HN to LN \((p = 0.0034)\), and from 13.3% to 17% of cortical area from 20k to 80k \((p = 0.0026)\). The effect of Era period on mean cortical cell size was significant, with size increasing 5% from 0.004 mm\(^2\) to 0.0042 mm\(^2\) in the newest \((p = 0.0232)\), but effect of N level and density were insignificant (Fig. 8B).

Metaxylem vessel number increased from 10.4 in the oldest Era period to 11.9 in the most recent Era period \((p = 0.0003)\), with no significant effect of N level or density (Fig. 9A). The median area of individual metaxylem vessels decreased 12.2% from 0.00947 mm\(^2\) in the oldest material to 0.00831 mm\(^2\) in the newest material \((p < 0.0001)\), with no significant effect of N level or density (Fig. 9B).

**Principal Component Analysis**

Principle component analysis of 15 crown root architectural and anatomical phenes revealed two components that explained 37% of the variation in root phenes (Fig. 10, abbreviations as given in Table 2). The anatomical phenes loaded onto the first component (PC1, 22% of the total variation), and the architectural phenes on the second (PC2, 15% of the total variation). ANOVA including N level, density, and Era as factors indicated no significant effects of these factors on the anatomical component. ANOVA for the architectural component had no significant effect of N level or Era, but the effect of density was significant \((p = 0.0041)\) with higher density plants have a lower score for PC2.
Simulation

In both the high density, high nitrogen (HDHN) environment and the low density, low nitrogen (LDLN) environment, the typical Old phenotype had the least shoot mass, while the Modern + RCA phenotype had the greatest shoot mass (Fig. 11). However, the relative ranking of the Old phenotype variants and the Modern phenotype variants changed. In the HDHN environment, Old + NRN ranked second in shoot mass, followed by Modern, then Old + Angle. In the LDLN environment, Old + Angle ranked second in shoot mass, followed by Modern, then Old + NRN. In the HDHN environment, shoot mass increased 15.8% from the Old phenotype to the Modern + RCA phenotype, and in the LDLN environment, shoot mass increased 12.9% from the Old phenotype to the Modern + RCA phenotype.

Discussion

Yield of the newest Era maize hybrids surpassed that of the oldest material in every N level and population density combination, and new Era hybrid yield was especially responsive to higher densities in high nitrogen relative to the older material, which confirms previous reports (Duvick et al., 2004). High density decreased leaf percent N which demonstrates the relationship between population density and N limitation induced by interplant competition. The greater yield of modern Era hybrids relative to older hybrids at greater densities and lower levels of nitrogen demonstrates the relatively greater nitrogen efficiency of the modern material, as suggested previously in a comparison of two genotypes (Tollenaar and Lee, 2002). To our knowledge, this is the first study to characterize the root system architecture and root anatomy of US hybrids produced over the past century. The hybrids of the newest Era period were marginally more shallow-angled, had fewer nodal roots, had a greater distance to branching, and slightly longer crown root laterals. Anatomically, newer Era hybrids had increased cell size and smaller yet more numerous metaxylem vessels. Many of these phene states were previously hypothesized to be optimal for water and nitrate uptake by the SCD ideotype (Lynch, 2013), except nodal root angle needs more careful consideration with regards to the vertical flux of soil sources and its influence on inter-and-intraplant competition. Changes in architectural and anatomical root phenotypes in US hybrids over the past 100 years are consistent with the evolution of the maize root system towards integrated phenotypes optimizing nitrogen acquisition efficiency in changing agronomic conditions.
The most recent material had brace roots that were 3° more shallow-angled and crown roots that were 7° more shallow-angled relative to the oldest material. However, all root angles in this study are steeper than 45° from horizontal, so may all be classified as relatively steep, especially considering that in the pioneering work of Weaver (1926) many of the angles of outer nodal roots range between 5° and 20° from horizontal (measured from Fig. 84 in that reference). More shallow-angled growth of axial roots has previously been shown to decrease interplant competition for phosphorus between neighboring root systems by decreasing the extent of root overlap (Ge et al., 2000), and this logic may be even more important for a more mobile resource like nitrate where depletion zones caused by root uptake will tend to be larger and more likely to overlap. Intraplant competition will be decreased when nodal roots are more shallow-angled because the proximity of roots of the same plant will also be increased, as shown with basal root growth angle in common bean (Ge et al., 2000; Rubio et al., 2001). Early in the growing season, availability of many soil resources, including nitrogen and phosphorus, are greater in the topsoil (Jobbágy and Jackson, 2001) so shallow-angled roots that explore the topsoil facilitate resource acquisition (Lynch and Brown, 2001). The root crown likely experiences the greatest degree of competition because of the great overall density of roots, so relatively shallow-angled roots in this region that go on to grow almost straight down may decrease competition in this zone, while co-optimizing shallow foraging and deep exploration. Nitrate leaches in agricultural systems so deep roots may be advantageous later in the season (Di and Cameron, 2002; Thorup-Kristensen, 2006), even though most leaching occurs during fallow periods. Similarly, in rain-fed environments, water may also become a deeper resource as the season advances (Asbjornsen et al., 2008). In common bean, shallow-angled roots that maximized the acquisition of limiting phosphorus, which is a shallow resource, early in the season supported greater plant shoot growth, greater subsequent photosynthesis, greater allocation to the root system, and therefore deeper rooting and greater yield during terminal drought (Ho et al., 2005). Maize with relatively shallow-angled roots that can still grow deeper may co-optimize the acquisition of several soil resources that have differing spatial and temporal availabilities.

The number of nodal roots will determine the overall intensity of soil exploration, and influence the carbon budget of the plant. The newest material had at least 1.6 fewer nodal roots than older lines. Phenes of the outer-most brace and crown whorls were measured in this study, however these are only 2 whorls of several. The overall decrease in the number of nodal roots could be greater if counted across all nodal root whorls. Recently, Saengwilai et al. (2014) demonstrated that maize root systems with fewer crown roots maximize N acquisition in low N soil because having fewer nodal roots decreases intraplant root competition, frees carbon and nutrients to be
used for other plant tissues, and may allow individual roots to grow longer and explore soil more effectively, which confirmed earlier simulation results (York et al., 2013). Previously, a hybrid released in 1998 was also found to have about one less nodal root per node than a hybrid released in 1959 and to have greater nitrogen stress tolerance (McCullough et al., 1994b). High density plantings may also create nitrogen stress, so fewer nodal roots may optimize N acquisition while decreasing interplant competition.

Distance from root tip to lateral emergence may be an important phene for P acquisition in common bean by reducing carbon costs and more efficiently exploring the soil (Miller et al. 2003). In this study we report a novel phene termed distance to branching, i.e. distance from the base of the root to the first lateral branch. For both brace and crown roots, distance to branching almost doubled from the oldest material to the most recent material, which implies indirect selection may have occurred for this phene. The maize root crown contains many nodal roots in close proximity and by the time the last whorl of crown roots and later brace roots emerge, the volume of soil in which the root crown grows is likely mostly depleted of resources. Delaying the emergence of lateral roots until they are farther from the stem may decrease the carbon costs of lateral root formation while having no or little effect on resource acquisition, which might allow greater carbon allocation to photosynthetic tissue, reproductive tissue, or roots in regions of soil containing greater availability of limiting resources.

Lateral root length has recently been shown to be an important phene in maize for nitrogen acquisition (Postma et al., 2014). The most recent material had longer lateral roots on crown roots, but there was no change in lateral root density. Consistent with the change in crown root lateral root length, Postma et al. (2014) demonstrated that fewer but longer lateral roots increase N acquisition when N is limiting, because nitrate is a mobile resource and longer lateral roots expand soil exploration while reducing intra-root competition. Thus, the longer crown root laterals observed in the most recent material are consistent with the hypothesis that modern root phenotypes have better N acquisition. Possibly the resources saved by the increased distance to branching in newer material could allow greater expenditures on lateral root length.

Cortical burden reflects the cumulative carbon costs of living cortical tissue (Jaramillo et al., 2013). Root cortical aerenchyma is formed when cortical cells senesce leaving behind air spaces which decreases root respiration (Fan et al., 2003). Simulation modeling demonstrated that reduced respiration and remobilization of nutrients after aerenchyma formation lead to increased root length and resource capture (Postma and Lynch, 2011a,b). In the field, maize recombinant inbred lines with greater aerenchyma formation had 800% more yield than lines with less aerenchyma lines.
under water stress (Zhu et al., 2010a), and 68% more yield in low nitrogen soil (Saengwilai et al., 2014a). In this study, all varieties had more aerenchyma in stressful conditions, such as LN and high density. However, the percent cortical aerenchyma did not change among Era periods, which could suggest undocumented tradeoffs for aerenchyma or a lack of diversity for this phene in the original material. Breeding for more and constitutively expressed aerenchyma may be an important target.

Larger cortical cells are hypothesized to decrease cortical burden by having a lower proportion of cytoplasm volume relative to vacuole volume and less respiratory and nutrient burden (Lynch, 2013). Roots of maize plants with large cortical cell size can respire 59% less than those with small cortical cell size, and can have up to 145% more yield in the field under drought stress (Chimungu et al., 2014a). Cortical cell size increased from the older to the most recent Era periods, which is consistent with indirect selection for large cortical cells through their positive influences on yield. Phenotypes that influence cortical burden may deserve special attention because if they have not been blind selected upon, then they may have more potential for yield gains relative to architectural phenotypes. However, Chimungu et al. (2014) found a positive relationship between cortical cell size and leaf cell size and speculate that genetic determination of plant cell size warrants further study because of possible functional tradeoffs in sizes of different cell types. Cortical cell file number (CCFN) is the number of layers of parenchyma cells in the root cortex, and reduced CCFN was associated with greater drought tolerance in maize (Chimungu et al., 2014b), however the material in this study had no differences in CCFN. Cortical phenotypes deserve more attention for their possible influences on soil resource acquisition in maize.

All else being equal, smaller diameter metaxylem vessels will decrease water flux (Lewis and Boose, 1995), even if an increase in their number leads to no change in the total area of metaxylem vessels. When water will be limiting during grain filling, conservation of soil water during the early season may increase yield (Lynch et al., 2014), as was observed in a study in which an older maize hybrid used more of the shallow water early in the season than a newer hybrid (Campos et al., 2004). A hybrid with relatively more and smaller diameter xylem vessels than a drought intolerant hybrid had greater yield under drought stress in the field (de Souza et al., 2013). Cavitation, where the water column is broken in xylem vessels, is another problematic water relationship, and a hybrid with more xylem vessels had less risk of cavitation (Li et al., 2009), so our observation of more but smaller metaxylem vessels in the newest Era material is consistent with potential drought and cavitation tolerance. Furthermore, fewer nodal roots in newer material
may also restrict total water flux and lead to more productive water use. Root anatomical phenes and their relations to water and nitrogen uptake deserve further attention (Lynch et al., 2014).

Changes in many of these phenes across Era periods were relatively small. However, even small marginal benefits of many phene states can compound over time to generate measurable performance differences. Duvick et al. (2004) also found small percent changes for many aboveground phenes in maize, and many of the changes were unintentional, or indirectly selected, as may have happened in the maize root system. In simulations to only 40 days, phenotypes with roots similar to the newest Era plants had the greatest shoot mass because of increased N uptake. Donald (Donald, 1968) proposed the concept of a crop ideotype as being useful for breeding and proposed that a cereal ideotype should be a weak competitor. Maize plants with relatively shallow-angled and fewer nodal roots are expected to experience less competition in pure stands than those with extremely steep-angled and many nodal roots, and to avoid root growth redundancy that may negatively affect plant performance (Zhang, 1999). The changes observed in root system architecture and anatomy are consistent with indirect selection for root systems that optimize N acquisition when fields are fertilized with N but high planting densities create competition that leads to individual plants experiencing some amount of nitrogen stress.

Previously research of root system and plant growth diversity among individuals of the wild maize ancestor, teosinte, and maize landraces offers further insight to the evolution of the maize root system. In a mesocosm screening of 195 maize landraces and 61 teosintes, several root architectural phenes were significantly different between landraces and teosintes, while fewer anatomical phenes were different between landraces and teosintes, which is a similar result as in this experiment where many architectural phenes differ among Era periods, but fewer anatomical phenes (Burton et al., 2013). Anatomical phenes and architectural phenes were also found to form distinct axes after principal component analysis, as in the current study. Architectural phenes may be more readily selected, or anatomical phenes might have stronger constitutive control. Landraces had fewer nodal roots but more seminal roots than teosintes (Burton et al., 2013), which might support a general decline in nodal root number as domesticated maize in the USA evolves in more controlled and less limiting environments, as observed in the current study. The reduction in branch points between teosinte and landraces (Burton et al., 2013) may also relate to changes in root system architecture that increase nitrogen acquisition efficiency through reduced lateral branching density (Postma et al., 2014). However, in a separate study within maize landraces, increased nodal root number was correlated to increased phosphorus (P) acquisition efficiency and also found to be greater in more traditional landraces (Bayuelo-Jiménez et al., 2011), which may demonstrate a
tradeoff between P (a immobile resource) acquisition and N (a mobile resource) acquisition, where more nodal roots are beneficial for growth in low P soil, but fewer nodal roots are beneficial for growth in low N soil. Nitrogen limitation is more common in US commercial maize fields than P limitation, which may be driving the decrease in nodal root number observed in the newest Era period. In six maize hybrids released between 1973 and 2000 in Northeastern China, root length density decreased in the top 20 cm of soil over time, but remained the same at greater depths (Chen et al., 2013), and though the researchers did not directly measure more elemental root system architectural phenes, these results are consistent with a decrease in nodal root number, especially in the youngest whorls that emerge in shallow soil (see Saengwilai et al. 2014). Evidence from the current study and others demonstrates possible evolution of the maize root system mediated by indirect selection towards phene states allowing greater N acquisition efficiency during the original domestication of maize, and its subsequent artificial selection in changing agronomic conditions.

Pioneer hybrids released in each decade from the 1930s to the 1990s have consistently had greater than 40% parentage from the landrace Reid’s Yellow Dent, and the total number of founders was about 60 during this same period (Smith et al., 2004). This diversity is only a fraction of what exists within the genus Zea, and the lack of genetic diversity may relate to a lack of diversity of root phenes. For example, the coefficient of variations (CVs) for nodal root number of the hybrids grown in the highest density and nitrogen levels in this study, landraces, and teosintes in the Burton et al. (2013) study are 0.178, 0.204, and 0.288, respectively. The CV for nodal root number decreases as groups of germplasm go from wild to original domestications to products of the past century’s breeding efforts, which supports that there is, in general, less phenotypic root diversity in elite maize hybrids used in the US than available than the Zea genus as whole. The introgression of extreme and moderate root phene states into elite backgrounds may be a desirable breeding goal for future studies.

The trajectories in phenotypic change in this experiment are apparent, and might allow extrapolation to future changes. Breeding has advanced such that more phene states may be introgressed to give rise to plant phenotypes that approach ideotypes for their target environments, whether through traditional breeding augmented with marker assisted selection, or genetic modification. Selection for yield and direct selection for specific shoot phenes states have contributed to genetic maize yield gains over the past century (Duvick et al., 2004; Tollenaar and Lee, 2006), but this study demonstrates changes in both root system architectural and anatomical phenes also occurred over the past 100 years. The changes in the maize root system observed possibly occurred through indirect selection through the effects of root phenes on yield, so direct
selection for positive acting root system phene states could contribute to future yield gains. However, breeding efforts must be based in mechanistic theory that includes how root and shoot phenes will interact and integrate to affect root system resource acquisition and overall plant performance (York et al., 2013). Newer material generated more yield in all agronomic contexts we investigated, and in all contexts, changes in root system phene states across Era periods were consistent with optimizing N acquisition. To our knowledge, the result of modern material from the Era set generating more yield in low nitrogen soil is novel, though McCullough et al. (1994b) had a similar result when comparing a hybrid released in 1959 with a hybrid released in 1988. Possibly, there is less conflict between breeding for high input and low input systems than previously recognized due to the intense competition in high density, high input systems causing some degree of soil resource stress. Breeding maize with root system architectural and anatomical phene states optimized for N acquisition in limiting conditions has the potential to contribute to more productive and more sustainable systems for both subsistence farmers in developing nations and commercial farmers in developed nations.

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Statement of Availability

Plant germplasm and transgenic material will not be made available except at the discretion of the owner and then only in accordance with all applicable governmental regulations. Novel materials described in this publication may be available for non-commercial research purposes
upon acceptance and signing of a material transfer agreement. In some cases such materials may contain or be derived from materials obtained from a third party. In such cases, distribution of material will be subject to the requisite permission from any third-party owners, licensors or controllers of all or parts of the material. Obtaining any permissions will be the sole responsibility of the requestor.

References


Figure 6-1. An image after being processed in *RSAJ* contains overlaid object measurements. The root crown is on the left, and the representative crown root on the right. A circular scale and sample ID label are in the top right of the frame. The green line measures scale diameter. The blue lines measure maximum width of focal whorl nodal roots, stem diameter, the distance between the two for angle calculations, and blue square count the number of roots in that whorl. The red lines measure representative nodal root diameter, distance to branching, the distance along the root within which lateral roots will be counted, and lateral root length. Red squares count the number of laterals.
Figure 6-2. Close-up views of SimRoot models parameterized as the average of the Old (left) and Modern (right) Era period phenotypes. The modern Era root system is marginally more shallow and has fewer nodal roots. More intense intra-root system competition occurs in the upper core of the root system, especially in the Old phenotype.
Figure 6-3. Leaf percent nitrogen (A) and grain yield on a per area basis (B) are presented. Dotted lines represent planting density with gaps between dots being proportional to differences in densities, with 20K, 40K, and 80K being 20,000, 40,000, and 80,000 plants ha$^{-1}$. Triangles are in low nitrogen (LN) and circles in high nitrogen (HN). Points represent the mean of the four varieties in that Era time period in the specific nitrogen and density combination and vertical lines the standard error. For grain yield, letters demonstrate groupings from Tukey HSD mean comparisons among Era time periods. Presence or absence of an asterisk next to a treatment in the legend indicates whether a treatment effect is significant or not.
Figure 6-4. Brace (A) and crown (B) root angle are expressed as degrees from horizontal such that larger angles are steeper. Dotted lines represent planting density with gaps between dots being proportional to differences in densities, with 20K, 40K, and 80K being 20,000, 40,000, and 80,000 plants ha\(^{-1}\), respectively. Triangles are in low nitrogen and circles in high nitrogen. Points represent the mean of the four varieties in that Era time period in the specific nitrogen and density combination, and vertical lines the standard error. Letters demonstrate groupings from Tukey HSD mean comparisons among Era time periods (no differences indicate an insignificant effect of Era period). Presence or absence of an asterisk next to a treatment in the legend indicates whether a treatment effect is significant or not, respectively.
Figure 6-5. Combined root number adds brace root number and crown root number. Dotted lines represent planting density with gaps between dots being proportional to differences in densities, with 20K, 40K, and 80K being 20,000, 40,000, and 80,000 plants ha\(^{-1}\). Triangles are in low nitrogen and circles in high nitrogen. Points represent the mean of the four varieties in that Era time period in the specific nitrogen and density combination, and vertical lines the standard error. Letters demonstrate a significant difference between the first and last Era periods based on a \(t\)-test (\(p = 0.01541\)) conducted after ANOVA demonstrated a significant effect of Era period. Presence or absence of an asterisk next to a treatment in the legend indicates whether a treatment effect is significant or not, respectively.
Figure 6-6. Distance to branching is the length along the nodal root from the stem to where lateral roots begin to emerge and is presented for brace (A) and crown (B) roots. Dotted lines represent planting density with gaps between dots being proportional to differences in densities, with 20K, 40K, and 80K being 20,000, 40,000, and 80,000 plants ha⁻¹. Triangles are in low nitrogen and circles in high nitrogen. Points represent the mean of the four varieties in that Era time period in the specific nitrogen and density combination, and vertical lines the standard error. Letters demonstrate groupings from Tukey HSD mean comparisons among Era time periods (no differences indicate an insignificant effect of Era period). Presence or absence of an asterisk next to a treatment in the legend indicates whether a treatment effect is significant or not, respectively.
Figure 6-7. Lateral root length was measured on 3 random lateral roots of the excised nodal root in each image and averaged before statistical analysis. Dotted lines represent planting density with gaps between dots being proportional to differences in densities, with 20K, 40K, and 80K being 20,000, 40,000, and 80,000 plants ha\(^{-1}\). Triangles are in low nitrogen and circles in high nitrogen. Points represent the mean of the four varieties in that Era time period in the specific nitrogen and density combination, and vertical lines the standard error. Letters demonstrate groupings from Tukey HSD mean comparisons among Era time periods (no differences indicate an insignificant effect of Era period). Presence or absence of an asterisk next to a treatment in the legend indicates whether a treatment effect is significant or not, respectively.
Figure 6-8. Percent cortical aerenchyma (A) and average cortical cell size (B) from anatomical sections from the field are presented. Dotted lines represent planting density with gaps between dots being proportional to differences in densities, with 20K, 40K, and 80K being 20,000, 40,000, and 80,000 plants ha⁻¹. Triangles are in low nitrogen and circles in high nitrogen. Points represent the mean of the four varieties in that Era time period in the specific nitrogen and density combination, and vertical lines the standard error. Letters demonstrate groupings from Tukey HSD mean comparisons among Era time periods (no differences indicate an insignificant effect of Era period). Presence or absence of an asterisk next to a treatment in the legend indicates whether a treatment effect is significant or not, respectively.
Figure 6-9. Number of metaxylem vessels (A) and the median area of each metaxylem vessel (B) from anatomical sections from the field are presented. Dotted lines represent planting density with gaps between dots being proportional to differences in densities, with 20K, 40K, and 80K being 20,000, 40,000, and 80,000 plants ha$^{-1}$. Triangles are in low nitrogen and circles in high nitrogen. Points represent the mean of the four varieties in that Era time period in the specific nitrogen and density combination, and vertical lines the standard error. Letters demonstrate groupings from Tukey HSD mean comparisons among Era time periods (no differences indicate an insignificant effect of Era period). Presence or absence of an asterisk next to a treatment in the legend indicates whether a treatment effect is significant or not, respectively.
Figure 6-10. Principal component analysis of crown root architectural and anatomical phenes conducted on raw data (n=261, must omit entire row when any measurement is missing). Points represent the scores of PC1 and PC2 for the individual root crown samples. Shades of gray of the inner circle color code for the Era periods with years in the legend being the beginning of that Era. Shapes surrounding the Era point code for population density. Labeled lines demonstrate the correlation of phene values to principal component scores (maximum correlation = .72).
Figure 6-11. Shoot mass as influenced by density and nitrogen levels and contrasting root phenes in the oldest hybrids relative to the newest. Plants were simulated in very high density (120,000 plants ha⁻¹) and high nitrogen (120K HN), and in low density (40,000 plants ha⁻¹) and low nitrogen (40K LN). Solid bars represent the means with lines being the standard error. Abbreviations at the bottom of the bar give the phenotype, as follows. The *Old* (O) phenotype has a steeper angle and a few more nodal roots than the *Modern* (M) phenotype. *Old* and *Modern* have low aerenchyma. *Old* + Angle (OA) is the same as the *Old* phenotype but with the same more shallow angle as the *Modern*, while *Old* + NRN (ON) is the same as the *Old* phenotype but with the same fewer nodal roots as the *Modern*. *Modern* + RCA (MR) is the *Modern* phenotype but with high aerenchyma.
Table 6-1. Sixteen commercially successful varieties from the past 100 years in the USA were used in this experiment. Year of release represents the prominent agronomic background in which the varieties were successful, while the breeding method is another important distinction.

<table>
<thead>
<tr>
<th>Era</th>
<th>Release</th>
<th>Breeding Method</th>
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<tbody>
<tr>
<td>1</td>
<td>Pre-1900</td>
<td>Open Pollinated Variety</td>
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<tr>
<td>1</td>
<td>1934</td>
<td>3-Way Cross</td>
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<tr>
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<tr>
<td>2</td>
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<tr>
<td>4</td>
<td>2011</td>
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Table 6-2. Table of root phenes, their abbreviations (Abr), and their descriptions, divided into architectural and anatomical phenes.

<table>
<thead>
<tr>
<th>Architecture</th>
<th>Abr</th>
<th>Description</th>
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<td>Ang</td>
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</tr>
<tr>
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<td>Number of nodal roots in whorl</td>
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<tr>
<td>Root Diameter</td>
<td>Diam</td>
<td>Diameter of nodal root</td>
</tr>
<tr>
<td>Distance to Branching</td>
<td>DTB</td>
<td>The distance from cut to first lateral emergence on nodal root</td>
</tr>
<tr>
<td>Lateral root density</td>
<td>LRD</td>
<td>Number of lateral roots within a centimeter of nodal root</td>
</tr>
<tr>
<td>Lateral root length</td>
<td>LRL</td>
<td>Average length of three lateral roots of nodal root</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anatomy</th>
<th>Abr</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross sectional area</td>
<td>RXSA</td>
<td>The total cross sectional area of a nodal root</td>
</tr>
<tr>
<td>Cortical cell area</td>
<td>CCA</td>
<td>The total area of a nodal root composed of cortical cells</td>
</tr>
<tr>
<td>Percent aerenchyma</td>
<td>%A</td>
<td>The percentage of the cortical area occupied by aerenchyma</td>
</tr>
<tr>
<td>Cell Files</td>
<td>CF</td>
<td>The number of cell files within the cortex</td>
</tr>
<tr>
<td>Cell Size</td>
<td>CS</td>
<td>The average area of individual cortical cells</td>
</tr>
<tr>
<td>Stele area</td>
<td>SA</td>
<td>The total area of the stele</td>
</tr>
<tr>
<td>Metaxylem vessel number</td>
<td>XV#</td>
<td>The total number of metaxylem vessels</td>
</tr>
<tr>
<td>Metaxylem area vessel</td>
<td>XVA</td>
<td>The average area of individual metaxylem vessels</td>
</tr>
<tr>
<td>Metaxylem area total</td>
<td>XVT</td>
<td>The total area of all metaxylem vessels</td>
</tr>
</tbody>
</table>
Table 6-3. ANOVA table of shoot measurements giving F-value and significance for all factors and factor interactions. ** p ≤ .01; * .01 < p ≤ .05; ns p > .05, not significant

<table>
<thead>
<tr>
<th>Factor</th>
<th>DF</th>
<th>Shoot Mass</th>
<th>% Leaf N</th>
<th>Total Leaf N</th>
<th>Yield Plant</th>
<th>Yield Ha&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>nlevel</td>
<td>1</td>
<td>10.97 *</td>
<td>379.46 **</td>
<td>364.43 **</td>
<td>110.06 **</td>
<td>142.84 **</td>
</tr>
<tr>
<td>density</td>
<td>2</td>
<td>505.52 **</td>
<td>54.61 **</td>
<td>194.84 **</td>
<td>451.89 **</td>
<td>147.46 **</td>
</tr>
<tr>
<td>era</td>
<td>3</td>
<td>1.47 ns</td>
<td>2.03 ns</td>
<td>2.78 *</td>
<td>52.55 **</td>
<td>68.70 **</td>
</tr>
<tr>
<td>nlevel:density</td>
<td>2</td>
<td>1.04 ns</td>
<td>5.37 **</td>
<td>9.56 **</td>
<td>6.33 **</td>
<td>74.86 **</td>
</tr>
<tr>
<td>nlevel:era</td>
<td>3</td>
<td>2.11 ns</td>
<td>0.62 ns</td>
<td>0.93 ns</td>
<td>3.07 *</td>
<td>7.66 **</td>
</tr>
<tr>
<td>density:era</td>
<td>6</td>
<td>1.35 ns</td>
<td>0.36 ns</td>
<td>1.04 ns</td>
<td>0.64 ns</td>
<td>7.95 **</td>
</tr>
<tr>
<td>nlevel:density:era</td>
<td>6</td>
<td>0.79 ns</td>
<td>1.83 ns</td>
<td>2.46 *</td>
<td>2.06 ns</td>
<td>4.13 **</td>
</tr>
</tbody>
</table>
Table 6-4. ANOVA table of crown root phenes giving F-value and significance for all factors and factor interactions. ** p ≤ .01; * .01 < p ≤ .05; ns p > .05, not significant. Phene abbreviations are as in Table 2, except amended with a crown root (CR) prefix and including stem (St) width at that node.

<table>
<thead>
<tr>
<th>Factor</th>
<th>DF</th>
<th>CR St Width</th>
<th>CR #</th>
<th>CR Diam</th>
<th>CR DTB</th>
<th>CR LRD</th>
<th>CR LRL</th>
<th>CR Ang</th>
</tr>
</thead>
<tbody>
<tr>
<td>nlevel</td>
<td>1</td>
<td>2.68 ns</td>
<td>0.12 ns</td>
<td>0.25 ns</td>
<td>2.61 ns</td>
<td>1.46 ns</td>
<td>0.83 ns</td>
<td>0.30 ns</td>
</tr>
<tr>
<td>density</td>
<td>2</td>
<td>23.77 **</td>
<td>2.35 ns</td>
<td>4.37 *</td>
<td>2.61 ns</td>
<td>0.16 ns</td>
<td>3.46 *</td>
<td>0.59 ns</td>
</tr>
<tr>
<td>era</td>
<td>3</td>
<td>7.03 **</td>
<td>1.77 ns</td>
<td>3.48 *</td>
<td>5.08 **</td>
<td>1.28 ns</td>
<td>8.15 **</td>
<td>9.13 **</td>
</tr>
<tr>
<td>nlevel:density</td>
<td>2</td>
<td>1.14 ns</td>
<td>0.54 ns</td>
<td>0.08 ns</td>
<td>0.04 ns</td>
<td>1.21 ns</td>
<td>1.11 ns</td>
<td>1.12 ns</td>
</tr>
<tr>
<td>nlevel:era</td>
<td>3</td>
<td>2.51 ns</td>
<td>1.34 ns</td>
<td>0.55 ns</td>
<td>1.24 ns</td>
<td>0.73 ns</td>
<td>0.92 ns</td>
<td>0.26 ns</td>
</tr>
<tr>
<td>density:era</td>
<td>6</td>
<td>0.75 ns</td>
<td>1.15 ns</td>
<td>0.27 ns</td>
<td>1.46 ns</td>
<td>0.48 ns</td>
<td>1.33 ns</td>
<td>0.48 ns</td>
</tr>
<tr>
<td>nlevel:density:era</td>
<td>6</td>
<td>0.65 ns</td>
<td>1.55 ns</td>
<td>0.89 ns</td>
<td>1.66 ns</td>
<td>0.63 ns</td>
<td>1.11 ns</td>
<td>0.98 ns</td>
</tr>
</tbody>
</table>
Table 6-5. ANOVA table of brace root phenes giving F-value and statistical significance for all
factors and factor interactions. ** p ≤ .01; * .01 < p ≤ .05; ns p > .05, not significant. Phene
abbreviations are as in Table 2, except amended with a brace root (BR) prefix and including stem
(St) width at that node.

<table>
<thead>
<tr>
<th>Factor</th>
<th>DF</th>
<th>BR St Width</th>
<th>BR #</th>
<th>BR Diam</th>
<th>BR DTB</th>
<th>BR LD</th>
<th>BR LL</th>
<th>BR Ang</th>
</tr>
</thead>
<tbody>
<tr>
<td>nlevel</td>
<td>1</td>
<td>29.71  *</td>
<td>37.45 **</td>
<td>16.31 *</td>
<td>28.92  *</td>
<td>3.71 ns</td>
<td>3.1 ns</td>
<td>2.41 ns</td>
</tr>
<tr>
<td>density</td>
<td>2</td>
<td>266.87 **</td>
<td>41.54 **</td>
<td>43.15 **</td>
<td>61.96 **</td>
<td>3.16 *</td>
<td>4.55 *</td>
<td>4.12 *</td>
</tr>
<tr>
<td>era</td>
<td>3</td>
<td>4.41 **</td>
<td>2.10 ns</td>
<td>0.90 ns</td>
<td>6.13 **</td>
<td>0.08 ns</td>
<td>1.41 ns</td>
<td>9.90 **</td>
</tr>
<tr>
<td>nlevel:density</td>
<td>2</td>
<td>2.25 ns</td>
<td>2.02 ns</td>
<td>1.08 ns</td>
<td>1.12 ns</td>
<td>0.76 ns</td>
<td>1.32 ns</td>
<td>0.86 ns</td>
</tr>
<tr>
<td>nlevel:era</td>
<td>3</td>
<td>1.55 ns</td>
<td>1.36 ns</td>
<td>0.23 ns</td>
<td>2.41 ns</td>
<td>0.12 ns</td>
<td>1.57 ns</td>
<td>0.27 ns</td>
</tr>
<tr>
<td>density:era</td>
<td>6</td>
<td>0.18 ns</td>
<td>0.45 ns</td>
<td>0.58 ns</td>
<td>0.29 ns</td>
<td>1.24 ns</td>
<td>0.54 ns</td>
<td>2.70 *</td>
</tr>
<tr>
<td>nlevel:density:era</td>
<td>6</td>
<td>0.36 ns</td>
<td>1.16 ns</td>
<td>1.24 ns</td>
<td>1.51 ns</td>
<td>0.80 ns</td>
<td>0.61 ns</td>
<td>0.69 ns</td>
</tr>
</tbody>
</table>
Table 6-6. ANOVA table of anatomy phenes giving F-value and statistical significance for all factors and factor interactions. ** $p \leq .01$; * $.01 < p \leq .05$; ns $p > .05$, not significant. Phene abbreviations as in Table 2.

<table>
<thead>
<tr>
<th>Factor</th>
<th>DF</th>
<th>RXSA</th>
<th>CCA</th>
<th>%A</th>
<th>CF</th>
<th>CS</th>
<th>SA</th>
<th>XV#</th>
<th>XVA</th>
<th>XVT</th>
</tr>
</thead>
<tbody>
<tr>
<td>nlevel</td>
<td>1</td>
<td>1.34 ns</td>
<td>19.13 *</td>
<td>71.93 **</td>
<td>0.79 ns</td>
<td>2.8 ns</td>
<td>0.94 ns</td>
<td>6.29 ns</td>
<td>0.53 ns</td>
<td>3.94 ns</td>
</tr>
<tr>
<td>density</td>
<td>2</td>
<td>1.05 ns</td>
<td>7.1 **</td>
<td>6.07 **</td>
<td>1.56 ns</td>
<td>1.64 ns</td>
<td>0.27 ns</td>
<td>2 ns</td>
<td>1.68 ns</td>
<td>1.77 ns</td>
</tr>
<tr>
<td>era</td>
<td>3</td>
<td>1.81 ns</td>
<td>2.92 *</td>
<td>1.71 ns</td>
<td>1.98 ns</td>
<td>3.23 *</td>
<td>1.68 ns</td>
<td>6.4 **</td>
<td>9.32 **</td>
<td>0.53 ns</td>
</tr>
<tr>
<td>nlevel:density</td>
<td>2</td>
<td>0.1 ns</td>
<td>0.34 ns</td>
<td>1.67 ns</td>
<td>0.06 ns</td>
<td>0.14 ns</td>
<td>0.06 ns</td>
<td>0.74 ns</td>
<td>0.91 ns</td>
<td>0.06 ns</td>
</tr>
<tr>
<td>nlevel:era</td>
<td>3</td>
<td>0.91 ns</td>
<td>0.13 ns</td>
<td>0.27 ns</td>
<td>0.27 ns</td>
<td>0.18 ns</td>
<td>0.48 ns</td>
<td>0.22 ns</td>
<td>0.83 ns</td>
<td>0.37 ns</td>
</tr>
<tr>
<td>density:era</td>
<td>6</td>
<td>0.32 ns</td>
<td>0.88 ns</td>
<td>1.2 ns</td>
<td>0.94 ns</td>
<td>1.19 ns</td>
<td>0.52 ns</td>
<td>1.76 ns</td>
<td>0.45 ns</td>
<td>0.57 ns</td>
</tr>
<tr>
<td>nlevel:density:era</td>
<td>6</td>
<td>0.2 ns</td>
<td>0.67 ns</td>
<td>0.49 ns</td>
<td>0.46 ns</td>
<td>0.63 ns</td>
<td>0.26 ns</td>
<td>0.84 ns</td>
<td>0.78 ns</td>
<td>0.86 ns</td>
</tr>
</tbody>
</table>
Chapter 7 General Conclusions

Maize is a globally important crop, and given the prevalent conditions of either nitrogen limitation or excessive use of fertilizers that lead to water and air pollution, the improvement of maize nitrogen use efficiency is an important goal for breeding programs. This dissertation sought to expand critical thinking on the functional utility of specific root phenes. A phene-based paradigm will be useful for considering how phenotypic variation translates to variation in nitrate uptake. The integration of root phenes for nitrate uptake undoubtedly has a large influence on the ability of maize to acquire nitrogen. Empirical work demonstrated that the different classes of the maize root system have different uptake kinetics that are affected by the environment. Simulation of roots systems with varying nitrate uptake kinetics and with different root system architectures demonstrated little interaction, probably because the cost of kinetics are not considered. In the future, more research on variation of kinetics among maize genotypes, and how this variation occurs through the expression of various nitrate transporters, will likely benefit crop improvement. Root phenes were demonstrated to vary within the maize root system in the field using intensive phenotyping that provides the most detailed analysis of the maize root system to date. Root phenes among nodes displayed additive integration and inclusion of multiple phenes in statistical models better accounted for variation in shoot mass and nitrogen content. Simulation modeling demonstrated substantial interaction between nodal root number, nodal root growth angle, and plant density. Fewer nodal roots that emerge later benefit maize growth in low nitrogen simulations, although the model fails to account for opportunity costs or risks associated with having fewer roots, such as herbivory and disease. The maize root system was demonstrated to have evolved towards states that correspond with greater nitrogen acquisition efficiency in maize hybrids from the past 100 years grown in the United States. Based on the results of this dissertation, the optimal ideotype of the maize root system for nitrate uptake has an intermediate number of nodal roots, intermediate growth angles, long laterals, increased distance from shoot to lateral root emergence, and high $I_{max}$. Intensive phenotyping and more complex statistical models are encouraged to reveal more about how plants deal with abiotic stress in general.
VITA
Larry Matthew York

RESEARCH INTERESTS

Root system form and function, plant competition, plant and microbe symbioses, simulation modeling, functional traits, next-generation phenotyping platforms

EDUCATION

<table>
<thead>
<tr>
<th>Institution</th>
<th>Degree</th>
<th>Major</th>
<th>Year</th>
</tr>
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<tr>
<td>Pennsylvania State University</td>
<td>Ph.D.</td>
<td>Ecology</td>
<td>2014</td>
</tr>
<tr>
<td>University of Kentucky</td>
<td>B.S.</td>
<td>Biology</td>
<td>2006</td>
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PUBLICATIONS

Journal Articles


FELLOWSHIPS, AWARDS, AND HONORS

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<th>Year</th>
<th>Award Description</th>
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<tr>
<td>2010–2013</td>
<td>Walter Thomas Memorial Scholarship, PSU</td>
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<tr>
<td>2011</td>
<td>College of Agricultural Sciences Competitive Grant, PSU</td>
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<tr>
<td>2010</td>
<td>Root Biology Center Scholarship, South China Agricultural University</td>
</tr>
<tr>
<td>2009</td>
<td>China Root Biology Fellowship, PSU</td>
</tr>
<tr>
<td>2008</td>
<td>Award for Excellence, PSU</td>
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<tr>
<td>2008</td>
<td>University Graduate Fellowship, PSU</td>
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PROFESSIONAL AFFILIATIONS

Agronomy Society of America
Crop Science Society of America
Soil Science Society of America
Ecology Society of America