Running head: Utility RCA NPK

Corresponding author:

Jonathan P. Lynch, Department of Horticulture, The Pennsylvania State University, University Park, PA, USA 16802, 814-863-2256, jpl4@psu.edu

Journal research area: Environmental Stress and Adaptation
Root cortical aerenchyma enhances growth of *Zea mays* L. on soils with suboptimal availability of nitrogen, phosphorus and potassium.

Johannes Auke Postma¹, Jonathan Paul Lynch¹,²
1 Department of Horticulture, The Pennsylvania State University, University Park, PA, USA 16802
2 For correspondence: E-mail jpl4@psu.edu
3 This research was supported by the National Science Foundation/BREAD [4184-UM-NSF-5380].
Abstract

Root cortical aerenchyma (RCA) is induced by hypoxia, drought and several nutrient deficiencies. Previous research showed that RCA formation reduces the respiration and nutrient content of root tissue. We used SimRoot, a functional-structural model, to provide quantitative support for the hypothesis that RCA formation is a useful adaptation to suboptimal availability of phosphorus, nitrogen and potassium by reducing the metabolic costs of soil exploration. RCA increased growth of simulated 40 day old *Zea mays* L. plants up to 55, 54 and 72% on low nitrogen, phosphorus or potassium soils respectively and reduced critical fertility levels by 13, 12 and 7% respectively. The greater utility of RCA on low potassium soils is associated with the fact that root growth in potassium deficient plants was more carbon limited than in phosphorus and nitrogen deficient plants. In contrast to potassium deficient plants, phosphorus and nitrogen deficient plants allocate more carbon to the root system as the deficiency develops. The utility of RCA also depended on other root phenes and environmental factors. On low phosphorus soils (7.5 μM), the utility of RCA was 2.9 times greater in plants with increased lateral branching density than in plants with normal branching. On low nitrate soils, the utility of RCA formation was 56% greater in coarser soils with high nitrate leaching. Large genetic variation in RCA formation and the utility of RCA for a range of stresses position RCA as an interesting crop breeding target for enhanced soil resource acquisition.

Keywords: *Zea mays* L., root cortical aerenchyma, RCA, phosphorus, nitrogen, potassium, deficiency, SimRoot, functional structural modeling, carbon economy
Introduction

Root cortical aerenchyma (RCA), i.e. enlarged gas spaces in the root cortex that form through either cell death or cell separation (Evans, 2003), is commonly known to form in response to hypoxia (Jackson, Armstrong, 1999). Improved oxygen transport is an important function of RCA formation in flooded soils where low oxygen availability may limit root respiration (Jackson, Armstrong, 1999). However, RCA also forms in response to a variety of other edaphic stresses, including phosphorus, nitrogen and sulfur deficiency and drought (Konings and Verschuren, 1980; Drew et al., 1989; Fan et al., 2003; Bouranis et al., 2003; Bouranis et al., 2006; Zhu et al., 2010). Thus it has been hypothesized that RCA formation has utility under a variety of edaphic stresses by reducing the metabolic costs of soil exploration (Lynch, Brown, 1998; Lynch, Brown, 2008). RCA, formed in maize by programmed cell death (Lenochová et al., 2009), reduces root nutrient content and respiration (Fan et al., 2003). Zhu et al. (2010) found that maize (Zea mays L.) genotypes with high RCA formation under drought had 5 times greater biomass production and 8 times greater yield than closely related genotypes with less RCA. In a previous study (Postma and Lynch, 2010), we presented quantitative evidence that remobilization of phosphorus from the root cortex and a reduction in maintenance respiration may allow plants to maintain greater growth rates in soils with low phosphorus availability. We hypothesized that these two functions, remobilization of nutrients and reduced respiration, could be important functions of RCA under other nutrient deficiencies as well. In this paper we evaluate the relative utility of RCA for the acquisition and utilization of nitrogen, phosphorus and potassium. We present, to our knowledge, the first evidence for a growth benefit of RCA formation in nitrogen and potassium deficient maize (Zea mays L.).

Nitrate is a mobile resource that in agroecosystems often leaches into the subsoil during the growing season (Di and Cameron, 2002). The dynamics of nitrate leaching present challenges to root systems which may have to capture nitrate from increasing depths. In contrast to nitrate, phosphorus and potassium are often more available in surface soil horizons and thus phenes that enhance 'topsoil foraging' may be more useful for acquisition of these nutrients (Lynch and Brown, 2001). RCA may release resources which allow the plant to invest in new root growth. The soil depth at which these investments occur relative to the availability of the nutrients at that depth may affect the utility of RCA. As a consequence, the nitrate leaching environment, as influenced by soil type and precipitation, may influence the utility of RCA in nitrogen deficient plants.

Maize forms RCA in response to nitrogen and phosphorus deprivation (Konings and Verschuren, 1980; Drew et al., 1989; Fan et al., 2003). It is unknown if RCA is formed in response to low potassium availability, however circumstantial evidence suggests that it might. Jung et al. (2009) show that ethylene mediates the response and tolerance to potassium deprivation in Arabidopsis thaliana L., stimulating root hair formation and primary root growth. Ethylene can be considered a general stress hormone mediating responses to drought (Schachtman and Goodger, 2008), and a number of nutrient deficiencies (He et al., 1992; Borch et al., 1999; Brown et al., 2003). Since ethylene is involved in signaling RCA formation (Drew et al., 2000) a possible increase in ethylene production due to potassium deficiency (Jung et al., 2009), may also result in increased RCA formation. Since RCA can form constitutively in maize plants under optimal conditions (Lenochová et al., 2009; Burton, 2010; Fan et al., 2003; Zhu et al., 2010), it may have value for potassium acquisition even if it is not induced by low potassium availability.
The formation of RCA depends on many factors including genetic, exogenous (environmental), and endogenous cues. As a result RCA formation may differ among and within root classes of the same plant, and may vary along the length of a root segment (Bouranis et al., 2006; Lenochová et al., 2009; Burton, 2010). Quantitative information on RCA distribution is sparse and difficult to quantitatively relate to exogenous or endogenous cues. In our previous simulation study on the benefit of RCA formation for plant growth on low phosphorus soils (Postma, Lynch, 2010), we kept RCA formation equal for all root classes and only varied it depending on the age of the root segment. Currently, more information on local RCA formation has become available from a study by Burton (2010) which allows us to present the first spatiotemporal reconstruction of RCA formation in different genotypes.

The utility of a phene may depend on interactions with other phenes in integrated phenotypes. For example long root hairs are more beneficial for phosphorus acquisition in roots with high root hair density (Ma et al., 2001), and in genotypes with shallow roots (Miguel, 2011). These phene synergisms may be important considerations in breeding crops with greater tolerance of edaphic stress. In this study we evaluate a potential synergism between lateral branching density and RCA formation.

Quantitative information about the function of root phenes and how that function depends on the expression of the phene, other root phenes, and environmental factors is scarce but important for breeders and may aid in the understanding of phenotypic diversity. With our simulations, we provide quantitative estimates for the utility of RCA in different genotypes grown under different environmental conditions.

Materials and Methods

We used SimRoot (Postma and Lynch, 2010), a functional-structural plant model (Vos et al., 2010), to simulate the utility of RCA formation in maize growing in diverse environments. We simulated RCA formation in three different maize genotypes and varied the phosphorus, potassium or nitrate availability in the soil. For the nitrate study, we simulated a loamy-sand and a silt-loam soil and varied precipitation to create 6 different leaching environments. We also evaluated a possible synergism between RCA formation and lateral branching density.

Model description

SimRoot simulates the 3D architecture and soil resource acquisition of a root system as it develops over time. The root system consists of roots of distinct root classes. Each root is represented by a growing number of root segments. This architectural component of SimRoot has been described in Lynch et al. (1997). SimRoot simulates shoot growth and photosynthesis, non-geometrically (Postma and Lynch, 2010) using LINTUL (Spitters and Schapendonk, 1990). Growth in SimRoot is based on a source-sink model in which carbon is partitioned using a set of rules which have been described in Postma and Lynch (2010). SimRoot simulates the nutrient uptake for each root segment and compares the total uptake to the optimal and minimal nutrient requirements of the plant. A nutrient stress factor is calculated when nutrient uptake falls below the optimal nutrient requirements. This stress factor influences the leaf area expansion rate and photosynthetic efficiency of the shoot negatively in a nutrient-specific manner (see below). Phosphorus uptake is simulated using the Barber-Cushman model (Itoh and Barber, 1983; Postma and Lynch, 2010), while potassium and nitrate uptake are simulated by linking (see below) SimRoot to the three dimensional hydrological model SWMS3D (Šimunek et al., 1995). We considered the Barber-Cushman model inadequate for simulating nitrate...
uptake, as this model does not simulate leaching of nitrate and cannot simulate root competition in three dimensions. On the other hand, SWMS3D is not able to simulate the narrow phosphorus depletion zones at submillimeter resolution, as does the Barber-Cushman model (Postma et al., 2008; Postma and Lynch, 2010), as the resulting large number of finite element (FEM) nodes would require excessive computation (Hardelauf et al., 2007). The coarse resolution of the SWMS3D grid may cause the narrow phosphorus depletion zones to be artificially enlarged. Since we used two different models for simulating nutrient uptake, each with its strength and weaknesses, we include a comparison of the two simulation modules and determined the effect of model choice on our results. We hypothesized that the Barber-Cushman model in comparison to SWMS3D would predict greater uptake of mobile nutrients (nitrate) but less uptake of immobile nutrients (phosphate and potassium).

RCA formation in maize is simulated for each root segment using empirical data from Burton (2010) who determined the percent RCA for different root classes and at different locations along the root. The model interpolated RCA formation between these locations. RCA formation is allowed to either reduce the nutrient content of the root segments (rereallocation function) or the respiration of the root segments (respiration function) or both, as is the case in actual plants (Fan et al., 2003). Reduction in nutrient content and respiration of the root segment are based on a regression between amount of RCA and nutrient content and root respiration of live plants as presented by Fan et al., (2003).

**Effects of nutrient deficiency on plant growth**

The nutrient stress factor was allowed to affect the potential leaf area expansion rate and light use efficiency independently. A negative impact of the nutrient stress factor on light use efficiency resulted in reduced carbon availability for growth. A negative impact on the potential leaf area expansion rate resulted in reduced sink strength of the shoot, and consequently greater carbon availability for root growth. In this way the nutrient stress factor functioned as a growth regulator altering root/shoot ratios. A nutrient-specific stress response curve was used to determine the effect of internal nutrient concentrations (Ni, Ki and Pi) on light use efficiency and potential leaf area expansion rate (Appendix A). Suboptimal Ki strongly reduces light use efficiency (Terry and Ulrich, 1973; Stamp and Geisler, 1980; Zhao et al., 2001), but does not affect the potential leaf area expansion rate (Cakmak et al., 1994). In contrast, suboptimal Pi strongly affects potential leaf area expansion rate, but has minor effects on light use efficiency of the leaves (Lynch et al., 1991). The Ni strongly affects both the potential leaf area expansion rate and light use efficiency (Sinclair and Horie, 1989; Uhart and Andrade, 1995).

**Linking SWMS3D to SimRoot**

We linked SWMS3D (Šimunek et al., 1995) to SimRoot (Postma and Lynch, 2010) in order to simulate nitrate uptake by the plant. SWMS3D is a three dimensional hydraulic simulation model that includes a solute transport model. It simulates water transport in the soil by solving the Richard's equation and solute transport by solving the convection-dispersion equation. SWMS3D includes a water extraction term in the Richard's equation (Richards, 1931) which can be used to simulate water uptake by roots. The solute transport model also includes an extraction term for nutrient uptake by roots. We calculated the water uptake by roots by dividing the potential transpiration equally over the root length of the root system, following Somma et al. (1998). Although this is a simplification, we consider this a valid approximation in wet soils with a relatively uniform water distribution, the environment that we simulated. We calculated nutrient uptake by the roots using Michaelis-Menten kinetics (Claassen and
Barber, 1974). Thus nutrient uptake becomes a function of the nutrient concentration in the profile, while nutrient concentrations in the profile depend on the uptake. We used a two step method to solve this mutual dependency, where the initial prediction was calculated using forward Euler and the final result was calculated using backward Euler (Šimunek et al., 1995). Stability of the results was verified by checking the nutrient balance of the whole system which remained within 1% accuracy.

In order to link SimRoot to SWMS3D, we matched the root nodes in SimRoot, spaced 0.5-1 cm apart, to the nodes of the 1 cm cubed FEM grid used by SWMS3D. To do so, we matched all the FEM nodes within a distance of \( \sqrt{3} \), i.e. the diagonal length of one FEM, to the root nodes. We distributed nutrient uptake of the root nodes over the nearby FEM nodes using a weighing factor \( (1/d)^3/(\sum(1/d)^3) \), where \( d \) is the distance between the nearby FEM nodes and the root node. We calculated the average nutrient concentration at the root node surface by averaging the nutrient concentrations of the nearby nodes using the same weighing factor. The weighting factor gives a greater weight to the nearest node in an effort to avoid artificially increasing the domain of soil exploration.

Mineralization

We added a simple, one pool, mineralization model as described by Yang and Janssen (2000) to SWMS3D and ran it for each FEM node. We varied mineralization parameters along the vertical dimension only but allowed three dimensional variation in soil water content to influence mineralization. Indirect interactions between the plant model and the mineralization model occurred, as local drying of the rhizosphere was allowed to affect mineralization, and changes in mineral N content due to mineralization were allowed to affect nutrient uptake rates.

Distribution of RCA formation within the root system

We assumed that RCA formation starts behind the cell elongation zone of a root and increases over time until a maximum is reached. Thus the greatest percentage of RCA per cross sectional area can be found close to the base of the root. This is in accordance with observations by Fan et al. (2003), but not with those of Bouranis et al. (2006), Lenochová et al. (2009) and Burton (2010) who observed lower RCA formation at the base of the roots and greater RCA formation in the middle root sections. We did not simulate low RCA formation in the basal parts of the root system, as RCA formation is only reduced in the first 5 cm of the root (Mano et al., 2006; Bouranis et al., 2006). This is a very small part of the total root length and forms a negligible effect (<2%) on the total RCA formation.

Within Zea mays L. there is significant genotypic variation in RCA formation and distribution of RCA in the root system (Fan et al., 2003; Burton, 2010). We used data from Burton (2010) for a high (w64a) and low RCA (H99) inbred genotype and a high RCA hybrid genotype (pioneer 35H56) and developmental data from Mano et al. (Mano et al., 2006) to simulate the temporal and spatial dynamics of RCA formation. We also simulated a high RCA reference phenotype in which a high percentage (39%) of the root cross sectional area of all root classes is RCA. This value of 39% RCA is the greatest amount of RCA formation reported in the literature (Fan et al., 2003).

Description of the genotypes

W64a and H99 are two inbred lines of maize (Zea mays L.) differing in RCA production, with w64a forming approximately 3 times more RCA than H99 (Burton, 2010). We assumed that both genotypes
had comparable vigor as the literature reports both greater and lesser biomass production for w64a (Silva and Gabelman, 1992; Kaeppler et al., 2000; Mickelson and Kaeppler, 2005). H99 has 15 degree steeper nodal roots than w64a, has thicker major axes and 1.5 times greater lateral branching frequency. Compared to hybrids, lateral extension is reduced in both inbred lines (Picard and Bosco, 2006). H99 has larger seed (0.28 g) than w64a (0.2 g) but lower seed phosphorus concentration, 0.36 %, instead of 0.48 % (Pletch-Rivera and Kaeppler, 2007).

The hybrid (pioneer 36H56) that we simulated forms a similar amount of RCA as w64a (Burton, 2010). This hybrid is more vigorous than the inbred lines, has longer lateral roots than inbred lines and more nodal roots. The thickness of the roots is intermediate to that of H99 and w64a. We did not have data on the steepness of the root system of this hybrid, and assumed that the crown roots had an angle of 70 degrees from horizontal, which is the median angle for crown roots in the NAM populations (Trachsel, University Park, unpublished). The NAM populations are a set of 5000 recombinant inbred lines which were obtained by crossing 25 very diverse inbred lines with one common parent, B73 (http://www.panzea.org/lit/germplasm.html).

We simulated 4 genotypes: a reference hybrid (pioneer 36H56), and two inbred lines W64a and H99. Since our reference genotype forms RCA in lateral roots, and since RCA formation in lateral roots is under discussion, we also simulated the reference genotype without RCA formation in lateral roots. In these simulations, we kept nutrient availability low, such that the approximate growth reduction of non-RCA plants was about 92%. This is a realistic level of stress on small-scale subsistence farms in many parts of the world (Lynch, 2007) and is, according to our previous results (Postma and Lynch, 2010) the stress level at which RCA formation has the greatest metabolic utility.

Phene-synergism between lateral branching density and RCA formation

Trachsel (University Park, unpublished) found significant genetic variation in branching density and length of lateral roots among maize genotypes. We simulated the extremes of this variation to study any possible synergisms between RCA formation and lateral branching density under low nitrogen and phosphorus availability.

Heterogeneity in resource availability

We varied nutrient availability with depth, with greater nutrient availability in surface strata (Appendix A, figure 7). Nitrate leaching was varied by simulating two different soils, a loamy sand and a silt loam, and three different precipitation regimes, 62, 124 and 248 mm in 40 days. A rainfall of 124 mm in 40 days corresponds to the rainfall in Rock Springs, Pennsylvania, during the first 40 days of the 2009 growth season, while 62 and 248 mm are half and double this rainfall.

Metabolic costs of root maintenance respiration

Nielsen et al. (1998) estimated that the carbon costs of root maintenance respiration in phosphorus deficient bean plants could be as high as 39% of daily photosynthesis. In our previous simulation study (Postma and Lynch, 2010) root maintenance respiration varied between 16 and 37 percent of the cumulative photosynthesis. These direct metabolic costs of root respiration are substantial on low fertility soils, however the opportunity costs of root maintenance respiration might be even greater. The opportunity costs of root respiration are the costs of the missed opportunity to invest carbohydrates in
root growth rather than respiring them (Lynch and Ho, 2005). Additional root growth would have increased nutrient uptake and thereby growth. We simulated maize plants with and without root maintenance respiration (without altering respiration caused by growth and nutrient assimilation) in order to estimate the opportunity costs of root maintenance respiration.

System description

We simulated the first 40 days of growth of a single plant which was a representative individual of a uniform, monoculture plant community with a between-row spacing of 60 cm and a within-row spacing of 26 cm. Aboveground competition was simulated by including a shading function (Postma and Lynch, 2010). Belowground, realistic root density was simulated by mirroring the roots at mid-distance between the simulated and the hypothetical neighboring plants (Figure 1). Root competition in SimRoot was a result of depletion of nutrients of neighboring roots. Root competition in the Barber-Cushman model was implemented as described by Postma and Lynch (2010).

Parameterization

Parameterization was based on empirical data only, we did not calibrate the model. Parameters used in this study, with the references for these parameters, can be found in appendix A of this publication.

Random number generator

SimRoot uses a random number generator to simulate natural variation in growth rates, direction and branching frequency. The model results are different when we simulate this variation than if we use average values because of the non-linearity of many processes (data not shown). The random number generator causes some variation in the results from different runs which is multiplied when calculating the RCA benefit, because this calculation involves data of two simulation runs. We repeated our runs 8 times to estimate this variation and show standard error bars for this variation in our graphs.

Runs

In total we ran 2200 runs on the PennState clusters lionxi and lionxj (http://rcc.its.psu.edu/hpc/systems). We varied:

1) percent RCA formation,
2) the functional utility of RCA, either I) a relocation or II) a respiration benefit, or III) both,
3) the availability of nitrate, phosphorus and potassium in the soil from low to sufficient,
4) soil texture: loamy sand and silt loam,
5) precipitation: 62, 124, and 248 mm over 40 days of growth
6) genotypes: inbred genotypes H99 and w64a and hybrid genotype 36H56
7) our ‘max RCA’ reference genotype with and without RCA formation in the lateral roots

10
8) our reference genotype with and without root maintenance respiration.

Results

RCA utility under different nutrient deficiencies

RCA had a positive effect on plant growth under suboptimal availability of nitrogen, phosphorus and potassium (Figure 2). The utility of RCA depended on the intensity of the nutrient deficiency and on the nutrient involved. At low to medium deficiencies (plant d.w. 30-100% of non-stressed), RCA formation had the greatest utility when potassium was limiting compared to nitrogen and phosphorus, while in strongly deficient plants (plant d.w. 5-30% of non-stressed), RCA formation had the greatest utility when phosphorus was limiting. The utility of RCA generally decreased with decreasing nutrient deficiency, but peaked at medium deficiency levels when potassium was limiting. RCA reduced critical soil nutrient levels, defined as the nutrient level below which growth was reduced, by 13% for N, 12% for P, and 7% for K (data not shown). Plants benefited most from reallocating nutrients and to a lesser extent from a reduction in respiration. However when potassium deficiency limited growth, reduced respiration was the most important benefit of RCA formation.

Utility of RCA formation in lateral roots

The model predicted larger benefits of RCA in plants that form RCA in lateral roots (Figure 3). This utility of RCA formation in lateral roots was strongest on low nitrogen and phosphorus soils. Both functions of RCA were equally affected by RCA formation in the laterals.

Utility of RCA formation in three different genotypes

We simulated the root architecture of three maize genotypes and their RCA formation (visualized in figure 4, for animated movie see appendix B). The utility of RCA formation in these genotypes is less than the utility in our reference genotype (Figure 3 and 5), which is understandable from the much reduced RCA formation in these genotypes (Figure 4). RCA formation increased growth of the high RCA genotypes, w64a and 36H56 more than the low RCA genotype, H99, except on low potassium soils where H99 had a greater growth response to RCA than w64a. Although RCA had greater effects on growth of w64a than on H99, the increase in RCA benefit was not proportional to the 3 fold increase in RCA in w64a compared to H99. RCA affected growth of w64a most on low nitrate soils while RCA affected growth of the other genotypes equally on low nitrate, phosphorus or potassium soils. These results cannot be totally explained by the simulation of the separate functions of RCA, suggesting that interactions between the functions exist. When simulating the separate functions of RCA, in soils with greater resource availability RCA sometimes had a small negative effect on growth (data not shown). The error bars show that stochasticity in root phenes other than RCA can cause large variation in the utility of RCA among individuals of a single genotype. Stochasticity was caused by variation in growth rates, growth directions and branching frequencies of individual roots.

Interactions between RCA formation and lateral root formation

We used our high RCA reference plant, which forms equal amounts of RCA in all roots including laterals (Figure 4), to simulate the utility of RCA formation under nitrate and phosphorus deficiency,
given different lateral branching densities. The model predicted that RCA formation is more beneficial in plants with greater lateral branching densities when grown under low phosphorus but not when grown under low nitrate (Figure 6). In soils with moderate nitrate availability, RCA benefited plants with normal lateral branching density the most. The RCA utility for plants with different branching densities was near equal in soils with low nitrate availability.

Influence of soil type and precipitation on nitrate leaching and the utility of RCA.

We simulated a loamy sand and a silt loam with three levels of precipitation to vary the intensity of nitrate leaching. As expected, nitrate leaching increased with increasing precipitation (Figure 7). This increase was greater in the loamy sand than in the silt loam. Plants benefited more from RCA formation in high leaching environments than in low leaching environments (Figure 8). This increase is not only caused by an increase in stress in these environments, but also exists when comparing the RCA utility in different soils across a range of stress levels (Figure 9).

Model comparison

Both the Barber-Cushman and SWMS3D models simulated very similar amounts of potassium uptake under high potassium availability (Figure 1 in Appendix C). However, the spatial-temporal distribution of uptake differed strongly between the models (Figure 2 in Appendix C). The uptake per root class and over time varied more in the SWMS3D model. The Barber-Cushman model simulated greater total uptake than the SWMS3D model under low potassium availability, which resulted in significantly more growth (Figure 3 in Appendix C). The steeper response curve of the SWMS3D model to potassium availability (Figure 3 in Appendix C), caused RCA to be more beneficial in the SWMS3D model (Figure 3 in Appendix C). In accordance to our hypothesis, the SWMS3D model predicted greater uptake of phosphorus while the Barber-Cushman module simulated greater uptake of nitrate (Figure 4 in Appendix C).

Estimation of costs of root maintenance respiration

Root maintenance respiration reduced plant growth on low fertility soils up to 72% in comparison to plants with no root maintenance respiration (Figure 10). The costs of root maintenance respiration were greatest for plants on low potassium soils. Root maintenance respiration did not affect growth on high fertility soils as plant growth was sink-limited, not source-limited. Under conditions of high soil fertility, reduced respiration only increased the carbon storage in the plants without affecting growth (data not shown).

Discussion

RCA formation is an adaptation to multiple nutrient deficiencies

RCA forms in response to suboptimal availability of nitrogen, phosphorus, sulfur, and water (Konings and Verschuren, 1980; Drew et al., 1989; Fan et al., 2003; Bouranis et al., 2003; Bouranis et al., 2006, Zhu et al., 2010). Our simulation results support the hypothesis that RCA formation may substantially benefit plants experiencing deficiencies of nitrogen and phosphorus and suggest that RCA could be beneficial under potassium deficiency as well (Figure 2). The results indicate that RCA may have the
The greatest utility on low fertility soils. However, RCA formation also decreased critical soil nutrient levels, defined as the soil fertility below which growth is reduced, by 13, 12 and 7% for nitrogen, phosphorus and potassium, respectively. This suggests that cultivars with high RCA formation under non-stressed conditions may allow farmers to use substantially less fertilizer. Reduction in fertilizer use may be greater than the numbers presented here, as soil available nutrients are only partly derived from fertilizers. We do not know if plants form RCA in response to potassium deficiency but propose that they may. It is common for stresses to occur simultaneously, however it is difficult to realistically simulate plant responses to simultaneous nutrient deficiencies (Dathe et al., 2011). In environments where multiple stresses may occur simultaneously (Lynch and St Clair, 2004; Rubio et al., 2003), trade-offs for nutrient acquisition strategies often pose challenges to the plant. For example shallow rooting which increases phosphorus acquisition may reduce drought tolerance (Ho et al., 2005). Our results indicate that RCA may be a phene that is beneficial for several nutrient deficiencies.

The utility of RCA was greater in plants that were moderately potassium deficient than in plants that were severely (<10% potential growth) potassium deficient. This decline is caused by a reduction in the utility of the respiration function. Increased availability of carbon from reduced respiration causes the plant to grow more roots. Root growth, however, also requires the investment of nutrients. The time that it takes for nutrient acquisition to compensate for these investments increases with decreasing soil fertility (Postma and Lynch, 2010). The plant is more nutrient deficient during this period, which may have detrimental effects on shoot growth. The decline in RCA utility in severely potassium deficient plants was less pronounced in phosphorus deficient plants and not observed in nitrogen deficient plants. In our previous simulation study of the utility of RCA in low phosphorus soils, for which we used homogeneous soil profiles, this decline in the utility of RCA was more pronounced (Postma and Lynch, 2010). The heterogeneous soil profiles used in the present study caused root growth, on average, to be more beneficial, as the opportunity to grow into soil domains with greater soil fertility existed. Actual plants may take advantage of soil heterogeneity by changing rooting depth (Zhu et al., 2005) and by root proliferation into areas with greater soil fertility (Borch, 1999; Hodge, 2004).

The relative importance of the two functions of RCA depends on the nutrient deficiency involved

Reallocation of nutrients is predicted by the model to be the more important function of RCA formation in nitrogen and phosphorus deficient plants (Figure 2). The importance of reallocating nutrients agrees with Robinson's (1990) calculations which show that reallocation of nutrients could be an important function of root cortical senescence in phosphorus deficient plants. Cortical senescence, like lysigenous RCA, is a form of programmed cell death (Deacon et al., 1986; Liljeroth and Bryngelsson, 2001). A reduction in respiration is more important in potassium deficient plants than in phosphorus or nitrogen deficient plants (Figure 2) because root growth is strongly carbon limited in potassium deficient plants, while this is not always the case for nitrogen and phosphorus deficient plants (Postma and Lynch, 2010). This carbon limitation of root growth in potassium deficient plants is caused by 1) the strong reduction in photosynthesis caused by the deficiency and 2) the lack of an adaptive response in carbon allocation between roots and shoots. While in nitrogen and phosphorus deficient plants photosynthesis may be reduced as well, deficiency of nitrogen and phosphorus cause increased carbon allocation to roots, at the expense of carbon allocation to shoots, which results in root growth being less carbon limited than it is under potassium deficiency. The strong carbon limited root growth in potassium deficient plants caused the opportunity costs of root maintenance respiration to be greater than the opportunity costs of root maintenance respiration of phosphorus and nitrogen deficient plants (Figure 10).
RCA formation in lateral roots has potential utility

Burton (2010) comments on the lack of RCA formation in lateral roots of non-stressed maize plants. However, Saengwilai (unpublished, University Park, 2010) did observe RCA formation in lateral roots of maize. Several scientists report RCA formation in lateral roots of other species in response to flooding (Laan et al., 1989; Gibberd et al., 2001; Thomas et al., 2005). According to our simulation results RCA formation in lateral roots would benefit nutrient deficient plants (Figure 3). These results suggest that lateral roots, despite their fineness, should not be ignored in RCA research.

RCA formation in three genotypes

Burton (2010) measured, in non-stressed plants, higher percent RCA formation in thicker root classes. Brace roots were an exception to the rule, as they were thick but formed little RCA. RCA formation in these brace roots may still have been in progress, however, since the oldest brace roots were only 7-10 days old. The correlation between root thickness and RCA formation is less clear in data from Mano et al. (2006) and Jaramillo-Velastegui (2011). Fan et al. (2003) show that under stress conditions relatively fine seminal roots can form high levels of RCA, up to 38% of the root cross-sectional area. Thicker root classes might not form more than 38% RCA under stressed conditions, as there is not much remaining living cortical area. Therefore we kept RCA formation equal in our reference plant which represents 'potential' RCA formation, but simulated actual RCA formation as measured by Burton (2010) in three different genotypes grown at high nutrient supply (Figure 4). We found that all three genotypes benefited from RCA formation when grown on low nitrogen, phosphorus or potassium soils (Figure 5). As expected from the reduced RCA formation, this benefit was much less than the benefit simulated for our reference plant (Figure 3). The difference in utility of RCA formation between the reference plant and the simulated genotypes may indicate the potential of breeding for RCA formation in these genotypes. However, trade-offs of RCA are currently not well understood (Postma and Lynch, 2010), and furthermore these genotypes may already form more RCA under stress. For example, low phosphorus availability increased RCA in seminal roots of w64a from 2 to 26% of the root cross-sectional area (Fan et al., 2003).

The reallocation and respiration functions of RCA in these genotypes depend on an interaction between the genotype and nutrient deficiency involved (Figure 5). We observed slightly negative growth responses to RCA when the functions were simulated independently on soils with medium fertility (Data not shown). These negative responses show that the two inbred lines did not allocate resources optimally under these specific conditions. In our simulation, nitrogen deficiency increased over time due to decreasing nitrate availability in the soil (caused by leaching and nitrate uptake by the plant). Adaptation to low nitrate availability by changing root shoot ratios takes time and therefore changes in carbon allocation must occur early if they are to result in growth benefits, especially considering that our simulations ended at 40 days after germination. Reallocation of nitrogen from lysed cells may cause the plant to be initially less deficient and thus respond with an attenuated adjustment of carbon partitioning between roots and shoots. However, the resulting greater shoot/root ratio may cause the plant to be more stressed during later growth stages. On the other hand, additional carbon for root growth requires an additional investment of nitrogen, which may cause the plant to be temporarily more stressed. Unless these investments in root growth pay off within a relatively short time, they may actually increase the negative effects of nutrient deficiency on growth (Postma and Lynch 2010). Thus both the reallocation and the respiration function have both positive and negative feedbacks in the model and when the functions are considered independently, negative feedbacks may exceed positive.
feedbacks. However, when, as in actual plants, both functions occur together, negative feedbacks are attenuated, and RCA has a net positive effect on plant growth. These negative feedbacks are caused by temporal disturbances in the nutrient homeostasis of the plant. Consequently, longer simulation times may change the negative responses to RCA into positive responses. Currently, our simulation times are restricted to 40 days, mainly because the datasets on which we based our parameterization do not extend beyond 40 days. We suggest that the study of older plants may be important in future research. Our results show that RCA is more beneficial if plants are able to invest their 'saved' resources more optimally. However, the optimal investment of resources is inherently complex as it depends on many factors and constraints of which only a few are included in this study.

Synergism between RCA and lateral root formation

H99, a low RCA forming genotype, benefits from RCA formation nearly as much as w64a, a high RCA forming genotype. H99 has a high lateral branching density of 15 laterals cm⁻¹ in contrast to the other two genotypes which have 10 laterals cm⁻¹ on average. RCA is more beneficial in plants with greater lateral branching density (Figure 6). Greater lateral branching density allows the plant to grow more root length, but only if it has the resources to do so. These resources can be made available through RCA formation. Greater lateral branching density is beneficial for phosphorus uptake, but it is less beneficial for nitrogen uptake, as it mostly leads to increased inter-root competition. Thus a positive interaction between lateral density and RCA formation for growth on low phosphorus soils exists, but this interaction does not exist for growth on low nitrogen soils (Figure 6). Phene synergisms, like the synergism between RCA formation and lateral root formation presented here, are an important consideration for breeders, and it is important to note that these phene synergisms may exist in one environment, but not in another.

RCA is more beneficial in environments with significant leaching

RCA formation was more beneficial in environments with greater leaching (Figure 8). In part the benefit of RCA in leaching environments is caused by increased nutrient deficiency in such environments. However, when comparing equally stressed plants, the utility of RCA was still greater in the loamy sand than in the silt loam (Figure 9). Therefore new root growth, made possible by RCA formation, was more beneficial when nitrate had leached to greater depth. This positive interaction did not exist in severely deficient plants (growth <30% of potential), which had reduced rooting depth. These phene by environment interactions may partly explain the observed genetic variation in RCA formation and may be considered by breeders in targeting specific environments.

Model choice did not affect conclusions

We simulated nitrate and potassium uptake using the SWMS3D model and phosphorus uptake using the Barber-Cushman model since we argued that SWMS3D is better for simulating mobile nutrients, while the Barber-Cushman model is better for simulating immobile nutrients (See material and methods). We predicted that SWMS3D would simulate greater uptake of phosphorus, an immobile nutrient, due to artificially increasing the depletion zones to 1 cm and that Barber-Cushman would simulate greater nitrate uptake, a mobile nutrient, as it cannot simulate nitrate leaching and root competition in 3 dimensions. Our results confirm our predictions and support our choice to use different models for different nutrients (Appendix C). We also predicted that both models would
simulate similar uptake of potassium which has intermediate mobility in the soil. Both models
simulated similar potassium uptake (Figure 2 and 4 in Appendix C), however, both models differed
strongly in the spatiotemporal distribution of potassium uptake (Figure 3 in Appendix C). The
potassium uptake rate was more constant over time and more equal among root classes when the
Barber-Cushman model was used. These results suggest that new modeling approaches are needed to
combine the strength of both models. Model choice affected our results on the utility of RCA formation
in maize plants for a number of potassium runs (Figure 4 in Appendix C) but did not effect our
conclusions that RCA is an adaptive phene to multiple nutrient deficiencies.

Model development

Our results indicate several areas in which structural functional plant models could be improved:

1) Parameterization for root development of older plants, in order to simulate full plant life cycles,
will be important for understanding the dynamics of physiological processes.

2) Root anatomy, which may affect whole plant physiology as shown in this report, is currently not
well understood in relation to root function and metabolic costs. It deserves more attention so
that it might be incorporated into functional structural models at greater detail.

3) Interactions between plants and other organisms such as mycorrhizae or neighboring plants are
important for understanding ecosystem functioning and may have consequences for the utility
of a root phene like RCA. It is important that the science community adopts an integrated and
quantitative approach in studying these interactions by developing models that can explain their
observations. The development of a functional quantitative plant-mycorrhizae model might be a
priority.

4) We compared two nutrient models and showed that each have their strengths and weaknesses. A
future challenge is to develop a model that combines the strength of both nutrient models.

Future directions for research

Our simulation results show that the formation of RCA can have utility for plant growth under multiple
nutrient deficiencies. These results indicate that small-scale changes in root anatomy may impact whole
plant physiology and growth. Root phenes, such as RCA, that influence the metabolic cost of soil
exploration may thus be key to understanding agroecosystem functioning and could be targeted in
breeding for nutrient efficient plants. We have shown that the utility of RCA depends on other root
phenes and on interactions with the environment. On soils with medium phosphorus availability, RCA
was 2.9 times more beneficial in plants with high lateral branching density compared to plants with
median branching density. Phene synergism was found for long and dense root hairs (Ma et al., 2001),
and long root hairs with shallow root growth angles (Miguel, 2011), whereas phene antagonism was
found between hypocotyl-borne and basal roots for phosphorus acquisition (Walk et al., 2006). We are
aware of few other studies that have considered phene interactions despite the possibility for strong
effects such as those found here. We hypothesize that RCA may be synergistic with phenes that are
beneficial for nutrient uptake but have high metabolic cost. For example, RCA may be synergistic with
the number of axial roots. More axial roots allow the plant to grow a larger root system, but if the
metabolic cost of the axial roots reduces lateral root growth, the phene may actually reduce plant
growth on low fertility soils. Thus there is an optimum number of axial roots which may be greater for plants that form RCA. In addition to metabolically costly root phenes, RCA may be synergistic with root phenes that position additional root growth in soil domains with greater fertility. For example RCA may be synergistic with shallow angles in low phosphorus soils where most of the phosphorus is available in the topsoil (Zhu et al., 2005). It may also be synergistic with root proliferation phenes in soils with low nitrogen. More research is needed on these phene interactions in integrated phenotypes. Integrated phenotypes need to be evaluated across a range of environments. RCA was more beneficial for maize growing on loamy-sand than on silt-loam and was more beneficial when precipitation was high on silt-loam soils. We hypothesized that RCA is more beneficial in environments with high nitrate leaching. Sulfur deprivation increases RCA formation (Bouranis et al., 2003, 2006). Low soil sulfur is usually the result of high sulfate leaching (McGrath and Zhao, 1995). RCA substantially increased growth of maize under drought (Zhu et al., 2010). We hypothesize that RCA may have special utility for deep soil resources like nitrate, sulfate and water. The utility of RCA for increasing the acquisition of these deep soil resources may depend on other root phenes such as steep branching angles and carbon allocation to growth at deep soil layers.

Figure 2 shows that a phene like RCA may have different utility at different fertility levels and that the relation between soil fertility and phene utility is not necessarily linear. As a consequence, the outcome of research in which high and low fertility levels are compared may depend on the actual fertility levels. Therefore quantitative approaches are required to evaluate phenes and their interactions with the environment. Simulation models are especially suitable for such quantitative analyses (Vos et al., 2010). We expect that functional structural models such as SimRoot will continue to provide new insights into plant function for some time.

Conclusions

We have provided quantitative evidence that RCA is an adaptive phene for multiple nutrient deficiencies. The utility of RCA for maize plants growing on soils with suboptimal availability of nitrate, phosphorus or potassium depends on multiple interactions between RCA formation, other root phenes and environmental factors. We found RCA to be synergistic with lateral root formation on low phosphorus soils. On low nitrogen soils, RCA may be more beneficial in environments with substantial leaching. This variability in the utility of RCA may explain the large variation observed for RCA formation (Fan et al., 2003; Burton, 2010). Undoubtedly, trade-offs for RCA formation, which are currently not well understood, contribute to this variability and therefore merit research. Functional structural models, like SimRoot, can be used to simulate this variation and thereby evaluate the utility of RCA in a specific genotype. We suggest that these models may become a valuable tool for evaluating breeding strategies that target a phene like RCA. Breeding for crop genotypes with enhanced soil resource acquisition will be an important strategy for reducing environmental pollution and decreasing agricultural reliance on fertilizer inputs (Lynch, 2007).

Acknowledgments

We acknowledge the important contributions of Dr. Annette Dathe to the development of both the Simunek and Barber-Cushman based modules that were used in this article. We are grateful for her assistance in solving the underlying mathematics of the Barber-Cushman model and her intensive involvement in the strategic planning, implementation and testing of the link between SimRoot and SWMS3D. We thank Dr. Maria Bredina Postma-Blaaauw and Dr. Eric Nord for their helpful review of
the manuscript.
Literature cited


Liljeroth, E, Bryngelsson, T (2001) DNA fragmentation in cereal roots indicative of programmed root


McGrath S, Zhao F (1995) A risk assessment of sulphur deficiency in cereals using soil and atmospheric deposition data. Soil Use Manage, **11**: 110-114

Mickelson SM, Kaeppler SM (2005) Evaluation of six mycorrhizal isolates for their ability to promote growth of maize genotypes under phosphorus deficiency. Maydica, **50**: 137–146


vesicular-arbuscular mycorrhizas on the carbon budget of common bean (*Phaseolus vulgaris* L.). New Phytol, **139**: 647-656

**Picard C, Bosco M** (2006) Heterozygosis drives maize hybrids to select elite 2,4-diacetylphloroglucinol-producing Pseudomonas strains among resident soil populations. FEMS Microbiol Ecol, **58**: 193-204

**Pletch-Rivera LA, Kaeppler SM** (2007) Phosphorus accumulation in maize grain is not influenced by xenia (*Zea mays* L.). Maydica, **52**: 151–157


**Richards LA** (1931) Capillary conduction of liquids through porous mediums. Physics, **1**: 318–333


**Silva ÁE, Gabelman WH** (1992) Screening maize inbred lines for tolerance to low-P stress condition. Plant Soil, **146**: 181-187


Figure legends

Figure 1: Side view of a simulated maize root system and its mirror image. The image shows how the model simulates a realistic root density by mirroring the roots back into the column.

Figure 2: The utility of RCA formation under different nutrient deficiencies. On the X-axis stress due to nutrient deficiency is expressed as the relative plant biomass at 40 days after germination compared to non-stressed plants. The RCA utility on the Y axis is expressed as growth increase due to RCA formation (Note the different scales). The first panel shows the overall benefit of RCA, the following panels show the benefit of RCA due to reallocation of nutrients and the benefit of RCA due to reduction in respiration. Each data point is an average of 2 repetitions.

Figure 3: The utility of RCA formation in roots when RCA only forms in the axial roots ("laterals without RCA") or when RCA forms in all roots ("lateral with RCA"). The utility of RCA formation is given in percentage increase in plant dry weight at 40 days after germination relative to the dry weights of plants simulated without RCA given in panel 4. Panels show utility on low nitrogen, phosphorus and potassium soils. Nitrogen, phosphorus, and potassium availability was such that yield reduction in plants without RCA was approximately 92%, corresponding to typical yield reduction of small-scale subsistence farmers. Panels as in figure 2. Error bars present standard error of the mean for eight repeated runs. Variation is caused by simulated stochasticity in root growth rates, growth directions and branching frequency.

Figure 4: Spatial map of RCA formation in simulated root systems at 40 days after germination. Colors show RCA formation as percentage of root cross-sectional area. The color range differed for the max RCA reference root system, which was rendered on a 0-40% scale, instead of a 0-15% scale. See text for detailed description of the differences between these genotypes which include variation in the steepness and number of major axis, the lateral branching density, the lateral root length and RCA formation. Roots have been dilated (~2 times) for better visibility and thus do not show true root thickness.

Figure 5: Comparison of the utility of RCA for different genotypes. See Figure 3 for description of the panels and error bars. Utility of RCA is much less than in figure 3, as RCA formation in these genotypes was much less (see figure 4).

Figure 6: Utility of RCA formation as affected by lateral root proliferation, under nitrogen and phosphorus deficiency. The legend shows three levels of lateral root proliferation: half, normal and double which correspond to 4, 8, and 16 laterals.cm\(^{-1}\). This range represents the full range in lateral branching density measured by Trachsel et al. (University Park, unpublished). Low and medium nitrate availability correspond to 11 and 64 kg.ha\(^{-1}\) residual nitrate in the top 60 cm (after fertilization) and low and medium phosphorus availability correspond to 5 and 7.5 \(\mu\)M in the buffered soil solution.

Figure 7: Nitrate leaching in a silt-loam and a loamy-sand soil given three different precipitation intensities. The legend lists total precipitation in mm over 40 days of growth. The '0 day' gray line shows the nitrate profile at the start of growth which had 21 kg.ha\(^{-1}\) residual N in the top 60 cm. Data is from simulations of the maximum RCA reference genotype (Figure 4).

Figure 8: Utility of RCA formation over 40 days of growth on a silt-loam and loamy-sand soil under different precipitation regimes. There was 21 kg.ha\(^{-1}\) residual N in the top 60 cm (See figure 7.)
simulated genotype was the maximum RCA reference genotype (Figure 4). See figure 3 for error bars.

Figure 9: Utility of RCA in two different soils, relative to the stress experienced due to nutrient deficiency. Axes as in figure 2.

Figure 10: Percent growth reduction (% plant dry weight) in 40 day old maize plants due to root maintenance respiration. Simulations of the reference genotype with (w) and without (o) maintenance respiration were compared using 100*(o-w)/o. Plants did not form RCA. X-axis as in figure 2.
Figure 1: Side view of a simulated maize root system and its mirror image. The image shows how the model simulates a realistic root density by mirroring the roots back into the column.
Figure 2: The utility of RCA formation under different nutrient deficiencies. On the X-axis stress due to nutrient deficiency is expressed as the relative plant biomass at 40 days after germination compared to non-stressed plants. The RCA utility on the Y axis is expressed as growth increase due to RCA formation (Note the different scales). The first panel shows the overall benefit of RCA, the following panels show the benefit of RCA due to reallocation of nutrients and the benefit of RCA due to reduction in respiration. Each data point is an average of 2 repetitions.
Figure 3: The utility of RCA formation in roots when RCA only forms in the axial roots ("laterals without RCA") or when RCA forms in all roots ("laterals with RCA"). The utility of RCA formation is given in percentage increase in plant dry weight at 40 days after germination relative to the dry weights of plants simulated without RCA given in panel 4. Panels show utility on low nitrogen, phosphorus and potassium soils. Nitrogen, phosphorus, and potassium availability was such that yield reduction in plants without RCA was approximately 92%, corresponding to typical yield reduction of small-scale subsistence farmers. Panels as in figure 2. Error bars present standard error of the mean for eight repeated runs. Variation is caused by simulated stochasticity in root growth rates, growth directions and branching frequency.

Figure 4: Spatial map of RCA formation in simulated root systems at 40 days after germination. Colors show RCA formation as percentage of root cross-sectional area. The color range differed for the max RCA reference root system, which was rendered on a 0-40% scale, instead of a 0-15% scale. See text for detailed description of the differences between these genotypes which include variation in the steepness and number of major axis, the lateral branching density, the lateral root length and RCA formation. Roots have been dilated (~2 times) for better visibility and thus do not show true root thickness.
Figure 5: Comparison of the utility of RCA for different genotypes. See Figure 3 for description of the panels and error bars. Utility of RCA is much less than in figure 3, as RCA formation in these genotypes was much less (see figure 4).

Figure 6: Utility of RCA formation as affected by lateral root proliferation, under nitrogen and phosphorus deficiency. The legend shows three levels of lateral root proliferation: half, normal and double which correspond to 4, 8, and 16 laterals.cm\(^{-1}\). This range represents the full range in lateral branching density measured by Trachsel et al. (University Park, unpublished). Low and medium nitrate availability correspond to 11 and 64 kg.ha\(^{-1}\) residual nitrate in the top 60 cm (after fertilization) and low and medium phosphorus availability correspond to 5 and 7.5 μM in the buffered soil solution.
Figure 7: Nitrate leaching in a silt-loam and a loamy-sand soil given three different precipitation intensities. The legend lists total precipitation in mm over 40 days of growth. The ‘0 day’ gray line shows the nitrate profile at the start of growth which had 21 kg.ha\(^{-1}\) residual N in the top 60 cm. Data is from simulations of the maximum RCA reference genotype (Figure 4).

Figure 8: Utility of RCA formation over 40 days of growth on a silt-loam and loamy-sand soil under different precipitation regimes. There was 21 kg.ha\(^{-1}\) residual N in the top 60 cm (See figure 7.) The simulated genotype was the maximum RCA reference genotype (Figure 4). See figure 3 for error bars.

Figure 9: Utility of RCA in two different soils, relative to the stress experienced due to nutrient deficiency. Axes as in figure 2.
Figure 10: Percent growth reduction (% plant dry weight) in 40 day old maize plants due to root maintenance respiration. Simulations of the reference genotype with (w) and without (o) maintenance respiration were compared using $100\times(o-w)/o$. Plants did not form RCA. X-axis as in figure 2.