How changing root system architecture can help tackle a reduction in soil phosphate (P) levels for better plant P acquisition

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Abstract
The readily available global rock phosphate (P) reserves may run out within the next 50-130 years, causing soils to have a reduced P concentration which will affect plant P uptake. Using a combination of mathematical modelling and experimental data we investigated potential plant-based options for optimising crop P uptake in reduced soil P environments. By varying the P concentration within a well-mixed agricultural soil, for high and low P (35.5 to 12.5 mg l$^{-1}$ respectively, using Olsen’s P index), we investigated branching distributions within a wheat root system that maximise P uptake.

Changing the root branching distribution from linear (evenly spaced branches) to strongly exponential (a greater number of branches at the top of the soil), improves P uptake by 142% for low P soils when root mass is kept constant between simulations. This causes the roots to emerge earlier and mimics topsoil foraging. Manipulating root branching patterns, to maximise P uptake, is not enough on its own to overcome the drop in soil P from high to low P. Further mechanisms have to be considered to fully understand the impact of P reduction on plant development.

Keyword index: modelling, plant nutrient uptake, rhizosphere, root architecture, *Triticum aestivum*
**Introduction**

Fertiliser prices are continuing to increase, following a dramatic rise and fall in 2008. The increased volatility in the price of nutrients is linked to the price of oil, and doubt about the limitation of rock P availability in the medium term, maybe outweighed by limitations in energy and sulphur to process rock phosphate. Further, there have been repeated and increasing warnings stating that the readily available global rock phosphate (P) reserves will become exhausted within the next 50-130 years (Déry & Anderson, 2007; Cordell, Drangert & White, 2009). Therefore careful use of this finite resource in agricultural systems is clearly warranted (Vaccari, 2009). This need to reduce our reliance on rock P may also become exacerbated by political control as the remaining reserves are highly spatially localised, being mainly owned by China, Morocco and the US, who together control 85% of the known global phosphorus reserves (Elser & Bennett, 2011).

P is typically applied in large quantities in most productive cropping systems (>20 kg P ha$^{-1}$), however, it is often used inefficiently with a large proportion of the added P subsequently becoming unavailable for plant P uptake or lost altogether. To achieve greater sustainability within agriculture requires new strategies that will either reduce the P demand of the crop or promote greater root recovery of the added P such that less fertiliser is required (Withers et al., 2014). This would reduce the negative aspects of P use in agriculture (e.g. eutrophication) as well as yielding greater economic returns for farmers. Repeated fertilisation over many decades can lead agricultural soils close to, or at, P saturated levels (Borda et al., 2011). While this increases organic and readily available P in the soil it also stimulates vertical loss down the soil profile and allows P to be readily released from particles when surface runoff enters freshwaters (Hartikainen, Rasa & Withers, 2010; Stutter et al., 2012). One mitigation strategy is therefore to “run down” soil P reserves by reducing P inputs relative to the amount of P offtake in the crop. To maintain yields, however,
necessitates that P is used more efficiently by the crop. It is therefore important to assess how crops will cope under a reduced P environment, and if that is not plausible, determine what plant-based options are available, for adapting to these conditions.

There are many potential strategies to help tackle the reduced P scenario, from changing the plant traits by targeted plant breeding (e.g. reduced seed P content, changes in root architecture), to altering the properties of the soil (Vance, Uhde-Stone & Allan, 2003; Lynch, 2007). Plants are estimated to take up less than 15% of the P added in the soil, and therefore an alternative method involves manipulating the chemistry and biology of the rhizosphere to make more of the added P available to plants (Qiu, 2010). As P is often highly immobile in soil, one method could be to adapt the root system architecture to obtain P more efficiently (Williamson et al., 2001; Ho et al., 2005).

Simulating P uptake by a growing root system using mathematical models enables us to capture a multitude of scenarios in less time and at significantly lower costs than via experimentation. However, the experimentation is essential to provide validation and parameters for the model. In this paper experimental data and model simulations are brought together to further advance the understanding of P uptake by plant root systems. Optimisation algorithms are used to further synthesise new knowledge from the models and to get the most out of the collected data. Although previous models have been developed to investigate the influence of root architecture on plant P acquisition (Ge, Rubio & Lynch, 2000; Lynch & Brown, 2001; Grant & Robertson, 1997), these studies followed a pseudo 3 dimensional approach (Lynch et al., 1997) that presents computational problems in up-scaling to the field level (Roose & Schnepf, 2008). A review of the current 3 dimensional models is well described in Dunbabin et al. (2013) providing strengths and weaknesses of each approach. Here we present an alternative approach to modelling P uptake: using an adaptation of the more efficient root system model of Roose et al. (2001) to simulate P uptake of a crop on a
field scale. This model is comparable to other density based root models (Dupuy, Gregory and Bengough, 2010). Roose et al. (2001) capture the nutrient depletion zone along all roots and scale up an analytical solution for a single ordered root to produce an accurate estimate for plant P uptake per soil surface area; extrapolating surface area to produce field scale results.

Wheat (*Triticum aestivum* L.) is a key crop for global food production, with total worldwide yields for 2012 estimated to be 652.17 Mt (USDA, 2013). In this study, the increasingly popular winter wheat cultivar variety 'Gallant' was used to provide the root parameters for the Roose et al. (2001) model. This model has been adapted so that different root structural patterns can be simulated and the optimal root branching structure that maximises P uptake determined. To check if a certain root structure will give adequate compensation, the effect of lowering the soil P concentration level will be assessed.

**Materials and Methods**

**Experimental collection of plant parameters**

**Plant root growth**

Given the variability of rooting within crop varieties (Středa et al., 2012), and the scarcity of studies quoting such basic root system characteristics, our own cultivar specific set of rooting parameters were produced (Table 1). In all experiments the soils were passed through a 5 mm sieve before use. All plants were grown in a greenhouse maintained at a minimum of 20°C, supplied with artificial lighting providing at least 16 hour days. Experiments were conducted in the UK winter, therefore the temperature and number of daylight hours rarely exceeded these values.

To measure the physical characteristics of the roots required by the model, seeds were planted to a depth of 1 cm in perspex rhizotrons (30 cm × 30 cm × 1 cm) filled with a Eutric
Cambisol sandy clay loam textured soil (Abergwyngregyn, UK) which had a high available P content due to repeated long term fertilisation (Olsen P = 33 mg l⁻¹ see Jones et al. (2004) for further details of the soil). This soil was maintained at 80% water holding capacity by watering three times a week. We used 2-dimensional rhizotrons as these have been shown to be representative of basic root architecture for cereal plants growing unconstrained (Hargreaves, Gregory & Bengough, 2009). The rhizotrons were tilted at a 30° angle to allow visualisation of the root system and measurement of root attributes: root growth of roots growing along the edge of the rhizotrons were measured by monitoring their progress with a ruler, and visible branching angles were measured using a protractor. It should be noted, however, the short length of the second order roots meant that measurement of their growth rate was not possible using this approach. At 21 days after emergence the plants were harvested. The roots were washed thoroughly by hand in distilled water, floated out on water in transparent plastic trays, and scanned using a flatbed scanner (Epson Perfection 4990 Photo). The diameter of each root order was then determined, using WinRhizo® software (Regent Instruments Inc., Canada). The inter branch distances, non-branching zone lengths and maximum root lengths were then measured manually for each root system using a ruler. To estimate root hair density and average lengths, 1 cm samples from the centre of each of these washed roots were mounted on slides in 50% glycerol and observed using a light microscope (Axioplan 2; Carl Zeiss Ltd, Cambridge, UK). The number of hairs protruding from each cm section of root as seen when mounted on microscope the microscope slide was doubled to account for half the root not being visible, and then used to define the root hair density for each root order. The length of the root hairs in these sections was measured using the microscope’s eyepiece graticule, and then the average for each root order was then used to define the root hair lengths in the model.
Rooting responses to P

A key component of the plant physiological response to P is the variation of root production (Drew, 1975). To ensure this would be factored into the model, an experiment was designed to measure the difference in rooting characteristics in low and high P soils. Seeds were incubated in aerated de-ionised water overnight at room temperature and then grown on moist tissue paper until the roots reached ≈5 cm. This represents the start time in the model. These seedlings were then planted in 50 ml centrifuge tubes each containing 55 g of either Morfa Cambisol (low P, Olsen P = 12.6 mg l$^{-1}$) or Eutric Cambisol (high P, Olsen P = 33.0 mg l$^{-1}$) soils (both Abergwyngregyn, UK), maintained at 80 % water holding capacity, and kept in a greenhouse (as previously described) for 10 days. Despite this being a small mass of soil, the plant available P supply remains significantly greater than the plant’s total P demand over such a limited timeframe (Table 2). As the model assumes the relationship of soil solution P to sorped P is at equilibrium, it was decided that using a soil high in native P that was already at equilibrium would provide better high-P model fits than applying soluble P fertiliser to a low-P soil, which would then perturb the sorption equilibrium. After 10 days the plants were harvested and the root systems were washed in water to remove the soil, excised from the remainder of the plant, dried to remove surface water with tissue paper and weighed to assess the differences in root mass between low and high P soil environments (Table 3). The same cultivation method was also used to produce plants with which to measure the impact upon inter-branch distance of order 1 branches in low and high P soils (Table 3): the inter-branch distance measured by scanning each root system using the flatbed scanner (Epson Perfection 4990 Photo) and then using the resulting images to measure the distance between each order 1 root branch on the seminal roots of each plant.

**Plant P demand**
To estimate plant P demand, wheat seeds were germinated on moist tissue paper until the roots had reached approximately 5 cm after which the seedlings were transferred to pots containing the high P Eutric Cambisol soil (150 g). Over the next 10 d, plants were sequentially harvested, washed to remove the soil, and dried at 85°C overnight. The plants were then dry-ashed (550°C, 16 h), the residue dissolved in 0.5 M HCl and then their P content determined according to the ascorbate/molybdate blue method of Murphy & Riley (1962).

**Soil tests**

The relationship between P in solution \( (c, \text{ mol/l}) \) and P held on the solid phase of soil particles \( (c_s, \text{ mol/kg}) \) is described by the soil buffer power \( (b) \),

\[
b = \frac{dc_{tot}}{dc_s}
\]

for

\[
c_{tot} = (c \cdot \phi) + (c_s \cdot \rho_b),
\]

where \( \phi \) is the soil’s volumetric water content \( (\text{dm}^3 \text{ dm}^{-3}) \), and \( \rho_b \) is the soil bulk density \( (\text{kg dm}^{-3}) \).

To determine \( b \) (a constant used within the mathematical model), \( c_s \) and \( c \) a sorption isotherm was measured (Barber, 1984). Using varying initial solution concentrations of \(^{33}\text{P}\)-labelled KH\(_2\)PO\(_4\) \( (0 \text{ to } 1 \text{ mM}; 1 \text{ kBq ml}^{-1}, \text{ American Radiolabeled Chemicals Inc., USA}), 5 \text{ ml of P solution was added to } 1 \text{ g air-dry soil, shaken (200 rev min}^{-1}, 24 \text{ h), centrifuged (16,000 g, 15 min), the supernatant solution mixed with the liquid scintilant Optiphase ‘Hisafe’ 3 (Perkin-Elmer, Boston, MA, USA), and }^{33}\text{P concentration (c) measured using a Wallac 1404 a liquid scintillation counter (Perkin-Elmer, Boston, MA, USA). The amount of P sorbed to the solid phase (c_s) was calculated by difference. A Langmuir isotherm was then fitted to the experimental data using SigmaPlot v11 (Systat Software Inc., San Jose, CA) to
enable calculation of $c$, $c_s$ and $b$ for each soil. This was done by using the middle of each Olsen P index band from DEFRA (2010) (Table 4) as the total P ($c_{tot}$) value for high and low P soils. The corresponding $c$, $c_s$ and $b$ values for that $c_{tot}$ on the Langmuir isotherm were used as the initial conditions in the model, with $b$ remaining fixed throughout the duration of the experiments.

Statistics applied to experimental data

To test whether means from experimental data are significantly different to each other a two tailed t-test was performed, where $p<0.05$ would yield a positive significance. For two means, $x_1$ and $x_2$, with corresponding standard deviations, $s_1$ and $s_2$, and sample numbers, $n_1$ and $n_2$, equation 3 calculates the value of $t$,

\[
  t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2} \left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}.
\]

The following assumptions are made; there are two independent samples, the data is normally distributed and the samples have the same variance. Once $t$ is known the degrees of freedom (calculated from $(n_1 - 1) + (n_2 - 1)$) is needed to produce a $p$ value which is then compared to the confidence interval, 0.05 for 5%. If $p < 0.05$ then the means are significantly different.

Phosphate uptake model

Nye and Tinker (1977) and Barber (1984) have previously modelled nutrient uptake for a single cylindrical root surrounded by an infinite extent of soil, where the nutrient concentration is equal to the farfield nutrient concentration away from the root. Due to nonlinearity in the root nutrient uptake boundary condition, Nye and Tinker (1977) and Barber (1984) were forced to solve the model numerically, which meant that adapting a
single root model to a more realistic root system was computationally expensive. Roose et al. (2001) and Roose & Kirk (2009) provide a fully explicit “approximate” analytical solution to the Nye-Tinker-Barber model which enabled a more realistic model that utilises a more complex root branching structure. In all four previous studies (Nye & Tinker, 1977; Barber, 1984; Roose et al., 2001; Roose & Kirk, 2009) the uptake of P by roots is represented by Michaelis-Menten uptake kinetics and a convection-diffusion model containing a linear diffusion equation with a nonlinear root surface uptake condition. The rate of convective transport of nutrients is assumed to be negligible relative to diffusion (Jungk & Classen, 1997; Roose et al., 2001; Roose & Kirk, 2009). For a complete solution of the convection-diffusion equations for P transport to plant roots see Roose & Kirk (2009). Roose et al. (2001) calculate the total uptake of nutrients given an initial set of parameters, which represent the nutrient concentration, water saturation and root parameters, such as length and radius. The analytical solution for the flux of nutrients $F_D(t; a)$ into a root of radius $a$ by Roose et al. (2001) is given by,

$$F_D = \frac{2F_m c}{K_m + c + L + (4cK_m + (K_m - c + L)^2)^{1/2}}.$$

with,

$$L = \frac{F_m a}{2\phi D} \ln \left(1 + 4e^{-\gamma} \frac{\phi D}{(\phi + b) a^2} t_D \right),$$

where $F_m$ represents the maximum rate of P uptake ($\mu$mol cm$^{-2}$ s$^{-1}$), $c$ is the far field concentration of P in pore water ($\mu$mol cm$^{-3}$), $K_m$ is the Michaelis constant ($\mu$mol cm$^{-3}$), $\gamma \approx 0.5772$ is Euler’s constant, $\phi$ is the water saturation (dm$^3$ solution dm$^3$ soil), $D$ is the diffusion coefficient of nutrient in pore water (cm$^2$ s$^{-1}$), $b$ is the soil buffer power (dimensionless) and $t_D$ represents time (days). The values of these parameters, taken from Roose et al. (2001), are presented in Table 5 and it is assumed that the farfield concentration of P is constant within the soil. The model calculates the uptake of P for one zero order root
To capture the effect of root hairs on nutrient uptake, we will apply the method of Leitner et al. (2010b) where 3 different models for nutrient uptake were considered. A dimensionless parameter $\alpha$ is calculated and depending on the morphological and physiological properties of the root hairs 3 scenarios occur. For $\alpha \sim 1$, a concentration gradient dynamically develops within the root hair zone, for $\alpha > 1$, the uptake by root hairs is negligibly small and for $\alpha < 1$, P in the root hair zone is taken up instantaneously. The dimensionless parameter $\alpha$ is given by,

\[
\alpha = \log_e \left( \frac{d l_{ni} F_m}{D K_m} \right) / \log_e \left( \frac{l_{ni}}{K_i} \right),
\]

where $d$ is dimensionless factor that distinguishes between solution culture and soil systems (in the soil culture $d=1$; in soil $d=1/(\phi + b)$), $l_{ni}$ is the distance between two root hairs on the $i_{th}$ order root (cm) and $K_i$ is the $i_{th}$ order root length (cm).

The value of $\alpha$ for zero, first and second order roots is 0.466, 0.703 and 1.477, respectively. For zero and first order roots $\alpha < 1$, which means root hairs effectively extend the root radius by the root hair length. For second order roots $\alpha > 1$, which means the roots hairs have a small uptake compared to the roots and are neglected. Experimental data showed root hairs appearing everywhere on all ordered roots and as a result, increased root radius occurred over the entire root length.

Equation (4) is used to construct a model for the nutrient uptake of a plant root system. The root system consists of a distribution of roots of radius $a$ and length $l$. Figure 1 shows the layout of the root structure where the top section of the root is labelled $l_b$ and the bottom section $l_n$, which are the non-branching zones. The main root is called 0 order, side branches of this are called 1st order and so forth. The root system branches by creating smaller side
roots between the non-branching zones \( l_b \) and \( l_a \), and this starts commencing when the original root reaches the length \( l_b+l_a \). Given a root of length \( l \), there are \( [(l-l_a-l_b)/l_n] \) branches, where \( l_n \) is the interval for each branching root.

Different order growing roots will have different radii \( a_i \), and will grow at different rates \( L_i(t) \). The elongation of roots of order \( i \) decreases with age and is described by,

\[
\frac{dl}{dt} = L_i = r_i \left( 1 - \frac{l}{K_i} \right),
\]

where \( l \) is the length of the root (cm), \( r_i \) is the initial rate of growth (cm d\(^{-1}\)) and \( K_i \) is the \( i_{th} \) order root length (cm).

The Roose et al. (2001) model uses a constant branching rate to define root architecture, thereby creating an even branching distribution. To change the root architecture we replace the constant value by a root branching distribution parameter, which interpolates between an even branching distribution and one which exponentially decreases in root length density down the soil profile. An exponential branching distribution is used where the same final volume of roots is grown; however, it creates a root system where top soil foraging is maximised (Varney et al., 1991). This also matches observations of root proliferation in top soils (0-30 cm) when fertilisers are strategically placed (McConnell, Sander & Peterson, 1985). The exponential branching distribution (\( G \), the number of roots per cm) is described by,

\[
G = Ae^{-Bt},
\]

where two variables define the branching structure, \( A \) (cm\(^{-1}\)) denotes the maximum density distribution (i.e. the maximum number per cm) and \( B \) (cm\(^{-1}\)) denotes how density decays towards the tip of the main root \( l \). For example, at a linear branching rate of 0.7 cm we set \( A=1/0.7 \) cm\(^{-1}\) and \( B=0 \) cm\(^{-1}\).
The branching points are calculated by first varying \( l \) between 0 and \( d \), where \( d \) is the length of the final branching zone along the main root. Secondly, the total area created by the curve in equation (8) from \( l=0 \) to \( l=d \) is calculated. Thirdly, a point \( l \) such that the area covered by the curve from \( l=0 \) to \( l=l_i \) is calculated to be equal to the total area divided by the number of branching roots. The next point \( l_2 \) is chosen such that the area created between the two points \( l_i \) and \( l_2 \) is the same as between 0 and \( l_i \). Finally, continuing this approach will generate an equal number of branching roots, but the distribution will be exponential rather than linear.

The two parameter family in equation (8) can be reduced to a single parameter if the total final length of the root system is kept the same. This simplifies the fitting process, discussed in section ‘Model validation and optimisation’, as fewer parameters reduce the search space and thus the computational time of the model. The method is described in the set of equations below, which begins with the total number of roots \( N_i \), which are in the length range \((0,d_i)\) for root order \( i \).

\[
\text{Eqn. 9} \quad \int_0^{d_i} Ae^{-Bl} dl = N_i.
\]

Simplifying and solving equation (9) for \( A \) produces,

\[
\text{Eqn. 10} \quad A = -\frac{N_i B}{e^{-Bl_i} - 1},
\]

which generates the root branching distribution \( G \) that conserves the final size of the root system, just in terms of the new variable \( B \).

\[
\text{Eqn. 11} \quad G = \frac{N_i B}{1 - e^{-Bl_i}} e^{-Bl}.
\]

The values of \( d_i \) are prescribed to be equal to 100 cm and 7.9 cm for the main root and order 1 root, respectively, and \( N_i \) equal to the number of roots for each given order calculated from the experimental data presented in Table 1. The chosen variable \( B \) will be bounded, such that at its minimum, 0 cm\(^{-1}\), the root branching is linear and at its maximum, 10 cm\(^{-1}\), the root
branching is exponential and almost all the side roots branch at the top of the branching zone. Figure 2 shows the root structure (with only 50 side roots for simplification) for the cases where $B$ is 0, 5 and 10 cm$^{-1}$ and the different initial branching scenarios can be clearly seen between Figure 2a ($B=0$ cm$^{-1}$) and 2c ($B=10$ cm$^{-1}$). The minimum branching distance measured from the experimental data (0.067 cm) was also set as the minimum branching distance in the model, i.e. at the upper bound when $B=10$ cm$^{-1}$. As we assume there is a constant P concentration within the soil, every root is therefore given their own depletion zone which does not overlap with others within the time frame.

For modelling purposes the growth angles of the roots in our experiments are not used, all other values in Table 1 are used in the model. This is due to the fact that the initial P concentration in the soil is constant, and roots will achieve the same uptake from any position; it is therefore sufficient to just calculate the time at which a root started growing. This simplification in the root system is justified by the comparison made in Leitner (2010a), where the P uptake from the roots in the Roose et al. (2001) model was shown to be comparable to the one of a 3D plant root system.

The second order roots are experimentally shown to grow where the density of root mass is greatest rather than in a linear or exponential distribution. The greatest density of second order roots on a first order branch was experimentally calculated to be 1.153 second order roots per mm. Therefore the second order roots were modelled such that there were a greater number of branches at higher density areas with the greatest density capped at 1.153 roots per mm. This distribution can be seen in Figure 3 where the position of the second order roots is affected by the exponential distribution of the first order roots. In the linear branching distribution case all of the root branches are constant whereas for the exponential branching distribution case, the majority of second order roots appear nearer the top of the plant as there is a greater density of roots there.
Results

Model parameterisation

The experimentally derived values for wheat root characteristics for 0, 1st and 2nd order roots are summarised in Table 1. Significant differences were apparent for all characteristics for the different root types, except for ‘root angle on lower ordered root’. We used these values to parameterise the model to estimate the P uptake for different root branching distributions in soil possessing two contrasting P contents, 35.5 mg l⁻¹ (high P) and 12.5 mg l⁻¹ (low P)(Table 4).

Experimental analysis showed that the biomass of roots grown in a low P soil was reduced on average by 45% in 10 day-old plants compared to those grown in a high P soil, and yielded a significant difference ($P < 0.05$; Table 3). However, the inter-branch distance for the emergence of first order roots was not significantly greater when the roots were grown in a high P environment ($P > 0.05$; Table 3). To capture this P-induced change in root architecture within the model, the simulation scenarios for the low P soil had the maximum root length for all order roots capped to match the experimental data. To determine the impact of this capping, simulations were undertaken with both reduced and constant root mass. The effects of a reduced root mass could present problems with current plant nutrition strategies, and perhaps placement of nutrients could produce greater yields (Randall and Hoeft, 1988).

Model simulations

Figure 4 shows the model predictions of plant P uptake across a range of P concentrations within the soil for the different root branching distributions. For a given line of constant branching distribution, there is a linear relationship between P concentration and P uptake ($R^2$...
due to the model being deterministic). However, for the line of constant P concentration, there is non-linear relationship between branching distribution and P uptake.

Three scenarios in particular were studied; a linear branching distribution in a low and high P soil and an exponential branching distribution in a low P soil. For each of these scenarios our model estimated the amount of P uptake by the whole root system (Fig. 5). In the high P soil, the model predicted that the plant would acquire 183\% more P than a plant grown in the low P soil. When the root branching distribution was changed from a linear to an exponential pattern the model predicted that this improved plant P uptake by 142\% in the low P soil. This represents a reduction of 14.5\% in comparison with plants grown in a high P soil with a linear branching pattern.

The results for cumulative P uptake for the 3 root branching scenarios over a 90 d crop growth period are shown in Figure 5a. The end time of 90 d was chosen as it gave suitable long term behaviour for wheat growth. For the majority of the time period, up to around 65 d, the exponential branching distribution in a low P soil (green-dashed) possessed the greatest P uptake even when compared with the linear branching distribution in a high P soil (red-solid). This is due to the fact that the side roots emerge earlier and therefore there is a greater surface area to enable earlier P uptake. After 65 d, the linear branching distribution in a high P soil catches up with and overtakes the exponential branching distribution in a low P soil and can take advantage of the rich P environment. The shape of the P uptake curve is defined by the branching distribution. In both linear root branching examples (red-solid and blue-dotted) there is smooth hinge shape curve, however in the exponential root branching example (green-dashed) a saturation growth curve is observed, which is expected as the root system grows to its full length.

With the negative effect of reduced root mass in the low P soil (Fig. 5b), the difference between the low and high P soil was magnified. Plant P uptake for the exponential
branching distribution in a low P soil (green-dashed) fell by 74% compared to when the root system growth was not capped (Fig. 5a) and matches a linear exponential branching distribution with an effective Olsen P index of 3.7 (39 mg l\(^{-1}\)). Changing from a linear to an exponential branching distribution improves P uptake by 151% in the low P soil, but this is a large decrease of 78% when compared with a high P soil using a linear branching pattern; which is expected given the large reduction in root mass.

Model validation and optimisation

The estimated P uptake from our model was compared with the experimental data collected for a root system grown in a high and low P environment (Table 2, Fig. 6). The parameter for the root branching structure, \(B\), was fit to minimise the sum of squares difference between our model and the experimental data. The estimated total plant P uptake fits well with experimental data within the initial 10 d of growth; for the comparisons, high P with \(B=1.5\) cm\(^{-1}\) and high P data, and low P with \(B=7\) cm\(^{-1}\) and low P data. The scenario for a low P soil with \(B=7\) cm\(^{-1}\) is not enough to capture the effects of the experimental high P uptake, because it is difficult to overcome the 45% reduced root mass and beyond the 10 day mark this difference is amplified.

Discussion

The important question that needs addressing is how alteration of root system architecture could (by breeding or genetic manipulation) produce greater P uptake. To that end, the model by Roose et al. (2001) has been adapted by introducing a parameter that changes the root branching distribution. Our model has two parameters that we will directly manipulate, the nutrient concentration in the soil \(c\) and the root branching distribution parameter \(B\). By
looking at the effect of changing the P level against the root branching distribution, by
altering \( c \) (Table 4) and \( B \), the P uptake is estimated.

Our study estimated the P uptake using our experimental soil and plant parameters found in Table 1. Our model is adapted from Roose et al. (2001) such that the branching density distribution is allowed to change from linear to exponential, to see the effects that root structure with different P concentrations in the soil, has on P uptake. Three scenarios were considered, a high and low P concentration level with a linear branching distribution and a low P concentration with an exponential branching distribution. In these scenarios the effect of reduced root mass in low P soils is considered, as seen in our experimental results.

The experimental P uptake (Table 2, Fig. 6) fits best with a weak exponential root branching distribution for P3 data, which can be seen for certain crops. A shift towards increased early lateral rooting has previously been shown experimentally to increase P uptake efficiency (Zhu & Lynch, 2004), and this scenario is successfully captured in the model. The strong exponential branching modelled here is however more aggressive than our data suggests and is currently seen within wheat root developmental plasticity. Perhaps breeding varieties to adopt this rooting strategy would be limited by carbon availability from photosynthesis. Although our model simulates a uniform soil P profile, that top soil foraging has been shown to be an essential component of plant P acquisition (Zhu, Kaeppler & Lynch, 2005), provides further emphasis upon the need to produce lateral roots early in the plant’s growth; helping to improve root-foraging strategies (Richardson et al., 2011). By modelling a non-uniform soil P profile (Roose and Fowler, 2004) a better fit to the data could be achieved, given necessary depth dependent data of available soil P. This is the subject of our follow on work which will be published separately.

Our model shows that changing the root structure of the plant, to produce more lateral roots earlier, has a positive effect on the uptake and can help plants survive in lower
phosphate environments. This is corroborated by previous experimental approaches (Zhu & Lynch, 2004). On average a 147% increase in P uptake is achieved from having a highly exponential root branching distribution over a linear one. However this positive increase is not enough to completely overcome the difference between a high and low P soil environment. Therefore, although increasing early lateral root production will enhance P uptake, other plant and fertiliser based strategies would be required to produce the required yields at low soil P levels. For example, an increase to all root lengths of all orders in combination with the exponential root branching distribution is sufficient, as only an 8% improvement is needed to match an exponential branching distribution in a low P soil, with a linear branching distribution in a high P soil (without accounting for the reduced root mass in a low P soil).

The exponential branching distribution however does provide greater early P uptake in low P soils when compared to linear branching root systems grown in high P (Figure 4a). Early growth, and yield size, have been shown to be most significantly correlated with early P uptake levels (Boatwright and Viets, 1966; Brenchley, 1929; Grant et al., 2001; Green et al., 1973), and greater early P uptake, and the corresponding early vigour seedlings display is also viewed by industry as insurance against problems which may occur in the growing period such as adverse weather conditions. Vigorous early growth also provides quicker soil surface cover, and therefore is useful in the reduction of soil erosion which can be a significant driver of environmental problems, and loss of P from agricultural systems (Pimentel et al., 1995). The diminished uptake that exponential branching in low P displays over linear branching in high P could still potentially impact final yields, where P-uptake from the environment is still required to augment grain filling (Boatwright and Hass, 1961; Grant et al., 2001; Mohamed and Marshall, 1979), and also to facilitate carbohydrate translocation into the ripening grain (Sutton et al., 1983). However, such a small difference in final P uptake
could potentially be met by a small targeted application of P late in the growing season, whilst still allowing for significantly lower application rates of P fertiliser than in current systems. The enhanced effectiveness of the exponential branching distribution provides an insight into the potential benefits possible from crop breeding (Figure 4a). The extent of the wheat root system already varies significantly between varieties (Středa et al., 2012), and plant breeding efforts have been made to use plant breeding to produce cultivars with an enhanced ability to acquire P (Gahoonia & Nielsen, 2004). Significant improvements in crop growth and output have been demonstrated to be possible from targeted breeding to improve varieties (Siddique et al., 1989), therefore a re-profiling of root branching distribution is potentially possible, and could drive an increase in crop P-acquisition. Additional and more rigorous experiments would need to be undertaken to properly validate possible improved root structures and their effects in high and low P soil. Given the variations in root system size present in commercially available wheat varieties (Středa et al., 2012), a targeted breeding programme has the potential to provide a range of root architectural variations which may prove to be more suited to low P soils. Furthermore, other parameters from Table 1, such as root hair dynamics, could be re-calculated to find possible differences between high and low P soils.

Due to the root structure being diminished in a low P environment we implemented the reduced root mass scenario. The difference between the high and low P soils generated a substantial 45% root mass decrease after 10 days which heavily affected the P uptake values in the low P environment. In a low P environment, targeting P close to early root growth (seed dressing or placement of fertiliser in bands 5 cm down from seed) is emphasised as even more essential due to the fact that the plant’s ability to search out P in a low P soil is severely limited by the smaller area of soil the root system can cover.
This paper provides modelling basics towards the development of whole plant
nutrient uptake models, by assessing what root structures are needed for given concentrations
of P in the soil to maximise plant P uptake.

Acknowledgements
We would like to thank the BBSRC and DEFRA (BB/I024283/1) for funding S. Payvandi, The Royal Society University Research Fellowship for funding T. Roose, K. C. Zygalakis was partially funded by Award No. KUK-C1-013-04 of the king Abdullah University of Science and Technology (KAUST), EPSRC and CORMSIS for funding J. Fliege and EPSRC Complexity DTC for funding J. Heppell, Defra, BBSRC, Scottish Government, AHDB, and other industry partners through Sustainable Arable LINK Project LK09136 for funding S. Payvandi, P. Talboys, D.L. Jones and T. Roose; and two anonymous reviewers for insightful comments that improved the manuscript.

References


Středa T., Dostal V., Horakova V., Chloupek O. 2012. Effective use of water by wheat varieties with different root system sizes in rainfed experiments in Central Europe. *Agricultural Water Management* 104: 203-209.


### Table 1

Experimental values for nine wheat root characteristics for 0, 1\(^{st}\) and 2\(^{nd}\) order roots used in the mathematical modelling. The only non-significant values are between the root angles for 1\(^{st}\) and 2\(^{nd}\) order roots.

<table>
<thead>
<tr>
<th></th>
<th>Units</th>
<th>0 order root</th>
<th>1(^{st}) order root</th>
<th>2(^{nd}) order root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth rate</td>
<td>mm day(^{-1})</td>
<td>15.83 ± 5.2(^a)</td>
<td>8.97 ± 2.6(^b)</td>
<td>4.00*</td>
</tr>
<tr>
<td>Inter-root branch distance</td>
<td>mm</td>
<td>n/a</td>
<td>3.64 ± 2.2(^a)</td>
<td>2.44 ± 1.3(^b)</td>
</tr>
<tr>
<td>Root diameter</td>
<td>mm</td>
<td>0.516 ± 0.090(^a)</td>
<td>0.229 ± 0.037(^b)</td>
<td>0.192 ± 0.049(^c)</td>
</tr>
<tr>
<td>Length of no branching zone</td>
<td>mm</td>
<td>43 ± 8(^a)</td>
<td>12.2 ± 3.4(^b)</td>
<td>n/a</td>
</tr>
<tr>
<td>Tip to root hair distance</td>
<td>mm</td>
<td>0.48 ± 0.15(^a)</td>
<td>0.0615 ± 0.037(^b)</td>
<td>0.376 ± 0.20(^c)</td>
</tr>
<tr>
<td>Root angle on lower ordered root</td>
<td>degrees</td>
<td>n/a</td>
<td>60.6 ± 9.0(^a)</td>
<td>63.8 ± 14.7(^a)</td>
</tr>
<tr>
<td>Number of root hairs on root</td>
<td>cm(^{-1})</td>
<td>202 ± 52(^a)</td>
<td>250 ± 63(^b)</td>
<td>444 ± 120(^c)</td>
</tr>
<tr>
<td>Root hair length</td>
<td>mm</td>
<td>0.59 ± 0.25(^a)</td>
<td>0.49 ± 0.13(^b)</td>
<td>0.43 ± 0.11(^c)</td>
</tr>
<tr>
<td>Length of root</td>
<td>mm</td>
<td>1000**</td>
<td>79</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Values represent means ± SD and those bearing the same alphabet are not significantly different within a row. *Result estimated from experimental data which is consistent with Pagès *et al.* (1989). **Result taken from Sylvester-Bradley *et al.* (1997).

**Placement:** Materials and Methods – Plant root growth – line 109.
Table 2 Experimentally-derived average P uptake (µmol plant\(^{-1}\)) measured over the 10 day growth period after sowing, for high and low P soil environments. After 10 days the P uptake values become significantly different, for a two tailed test with P<0.05.

<table>
<thead>
<tr>
<th>Days after sowing (initial root length was between 10 and 15cm over 3 roots)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low P average uptake (µmol P plant(^{-1}))</td>
<td>0(^a)</td>
<td>0.058(^a)</td>
<td>0.14(^a)</td>
<td>0.37(^a)</td>
<td>0.79(^a)</td>
<td>1.3(^a)</td>
<td>2.1(^a)</td>
</tr>
<tr>
<td>Standard Deviation (µmol P plant(^{-1}))</td>
<td>n/a</td>
<td>0.19</td>
<td>0.35</td>
<td>0.34</td>
<td>0.37</td>
<td>0.54</td>
<td>0.49</td>
</tr>
<tr>
<td>High P average uptake (µmol P plant(^{-1}))</td>
<td>0(^a)</td>
<td>0(^a)</td>
<td>0.12(^a)</td>
<td>0.70(^a)</td>
<td>1.5(^a)</td>
<td>2.1(^a)</td>
<td>3.2(^b)</td>
</tr>
<tr>
<td>Standard Deviation (µmol P plant(^{-1}))</td>
<td>n/a</td>
<td>0.051</td>
<td>0.28</td>
<td>0.24</td>
<td>0.26</td>
<td>0.12</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Means bearing the same alphabet are not significantly different within a column.

Table 3 The average inter-root branching distances of first order roots and masses of fresh weight roots for high and low P soil environments. The average root mass was significantly different between high and low P, whereas the average inter-root branching distance was not.

<table>
<thead>
<tr>
<th></th>
<th>Low P</th>
<th>High P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average inter-root branching distance (mm)</td>
<td>$4.2 \pm 2.4^a$</td>
<td>$3.7 \pm 1.7^a$</td>
</tr>
<tr>
<td>Average root mass (mg per plant)</td>
<td>$586 \pm 141.7^a$</td>
<td>$313 \pm 117.1^b$</td>
</tr>
</tbody>
</table>

Values represent means ± SD and those bearing the same alphabet are not significantly different within a row.

Table 4 Relationship between the DEFRA (2010) agronomic index values for available soil P measured using the Olsen NaHCO$_3$ extract method and actual levels in the soil and soil solution ($P_{sol}$).

<table>
<thead>
<tr>
<th>DEFRA agronomic index value</th>
<th>$P$ (mg l$^{-1}$)</th>
<th>$P$ (mmol l$^{-1}$)</th>
<th>$P_{sol}$ = $c$ (μmol l$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index 0 (very low P)</td>
<td>0-9</td>
<td>0 – 0.2903</td>
<td>0 – 12.3</td>
</tr>
<tr>
<td>Index 1 (low P)</td>
<td>10-15</td>
<td>0.3226 – 0.4839</td>
<td>13.7 – 20.5</td>
</tr>
<tr>
<td>Index 2 (moderate P)</td>
<td>16-25</td>
<td>0.5161 – 0.8065</td>
<td>21.9 – 34.2</td>
</tr>
<tr>
<td>Index 3 (high P)</td>
<td>26-45</td>
<td>0.8387 – 1.4516</td>
<td>35.6 – 61.6</td>
</tr>
</tbody>
</table>

$P_{sol}$ is equivalent to the concentration of nutrients in pore water $c$ and is dependent upon the soil buffer power $b$ and the water saturation ($\phi$).

Placement: Materials and Methods – Soil tests – line 190.
Table 5 Soil and nutrient uptake parameters, with values and units.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\phi$</td>
<td>Soil volumetric water content</td>
<td>0.3</td>
<td>L solution L soil$^{-1}$</td>
</tr>
<tr>
<td>$D$</td>
<td>P diffusion coefficient in pore water</td>
<td>$0.3 \times 10^{-5}$</td>
<td>cm$^2$ s$^{-1}$</td>
</tr>
<tr>
<td>$b$</td>
<td>P buffer power in soil</td>
<td>239</td>
<td>-</td>
</tr>
<tr>
<td>$F_m$</td>
<td>Maximum rate of root P uptake</td>
<td>$3.26 \times 10^{-6}$</td>
<td>$\mu$mol cm$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Euler’s constant</td>
<td>0.5772</td>
<td>-</td>
</tr>
<tr>
<td>$K_m$</td>
<td>Michaelis constant for root P uptake</td>
<td>$5.8 \times 10^{-5}$</td>
<td>$\mu$mol cm$^{-3}$</td>
</tr>
</tbody>
</table>

Figure Legends

Figure 1 Branching structure of a root system, with non-branching zones $l_a$ and $l_b$, and inter-root branch distance $l_m$. The main root, order 0, branches order 1 roots which in turn branch order 2 roots.

Figure 2 The simulated root structure (with only 50 order 1 roots for simplification) for 3 different branching distributions; a) shows a linear branching distribution ($B = 0 \text{ cm}^{-1}$), b) shows a slight exponential distribution ($B = 5 \text{ cm}^{-1}$), and c) shows a strong exponential distribution ($B = 10 \text{ cm}^{-1}$).

Figure 3 The root distribution of order 2 roots; a) shows the distribution of order 2 roots for a linear branching distribution of order 1 roots, and b) shows the distribution of order 2 roots for an exponential distribution of order 1 roots. The greater the exponential distribution the denser the order 2 roots become.

Figure 4 Model estimates for whole plant P uptake ($\mu$mol P plant$^{-1}$) for different branching distributions ($B$) and initial soil P concentrations. At $B = 0$ we have a uniform branching distribution and for increasing values of $B$ we have more concentrated branching at the top of the soil profile.

Figure 5 Predicted cumulative plant P acquisition for three root branching scenarios, a linear branching distribution in a high and low P soil and an exponential branching distribution in a low P soil; Panel (a) shows P uptake when the final volume of roots is conserved, while panel (b) shows P uptake where there is a 45% reduced root biomass after 10 days for the low P scenarios.
Figure 6 Experimental and model values for the cumulative uptake of P by wheat seedlings over a 10 d period when grown in high and low P soil for a range of root branching distributions. The model values comprise of, a high P soil with a weak exponential distribution ($B = 1.5 \text{ cm}^{-1}$), and a low P with a strong exponential distribution ($B = 7 \text{ cm}^{-1}$).