

Enhanced zinc uptake by rice through phytosiderophore secretion: a modelling study

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ABSTRACT

Rice (*Oryza sativa* L.) secretes far smaller amounts of metal-complexing phytosiderophores (PS) than other grasses. But there is increasing evidence that it relies on PS secretion for its zinc (Zn) uptake. After nitrogen, Zn deficiency is the most common nutrient disorder in rice, affecting up to 50% of lowland rice soils globally. We developed a mathematical model of PS secretion from roots and resulting solubilization and uptake of Zn, allowing for root growth, diurnal variation in secretion, decomposition of the PS in the soil, and the transport and interaction of the PS and Zn in the soil. A sensitivity analysis showed that with realistic parameter values for rice in submerged soil, the typically observed rates of PS secretion from rice are sufficient and necessary to explain observed rates of Zn uptake. There is little effect of diurnal variation in secretion on cumulative Zn uptake, irrespective of other model parameter values, indicating that the observed diurnal variation is not causally related to Zn uptake efficiency. Rooting density has a large effect on uptake per unit PS secretion as a result of overlap of the zones of influence of neighbouring roots. The effects of other complications in the rice rhizosphere are discussed.

Key-words: cereal; micronutrient; mugineic acid; rhizosphere; solubilization.

INTRODUCTION

Zinc (Zn) deficiency in rice plants affects up to 50% of the lowland rice area globally (White & Zasoski 1999; Dobermann & Fairhurst 2000; Kirk 2004) and it is also often deficient in the diet of rice-eating populations (IRRI 2006). It is a particular problem in rice because of the biogeochemistry of submerged soils, where reducing conditions can lead to Zn being immobilized in highly insoluble forms (Kirk 2004). It is often associated with high soil organic matter content, high soil pH and high bicarbonate concentrations, as for example in the calcareous soils of the Indo-Gangetic

plain. But it is also widespread in young, perennially wet soils with weak profile development, where the Zn is in primary minerals or other insoluble forms formed under reducing conditions. There is currently renewed interest in rice Zn relations because of efforts to breed rice for high micronutrient contents for human nutrition (IRRI 2006).

Linked to this widespread occurrence, the rice germplasm contains large differences in tolerance to Zn-deficient soils (Quijano-Guerta *et al.* 2002; Wissuwa, Ismail & Yanagihara 2006). The mechanisms of tolerance are not well understood, but they appear to involve root growth attributes and ability to solubilize Zn in the rhizosphere (Kirk & Bajita 1995; Hoffland, Wei & Wissuwa 2006; Arnold *et al.* 2010; Widodo *et al.* 2010). In particular, there is evidence for the involvement of phytosiderophore (PS) secretion from the roots and uptake of PS-Zn complexes. Widodo *et al.* (2010) found enhanced secretion of the PS deoxymugineic acid (DMA) by a rice line from a population used to identify quantitative trait loci associated with low Zn tolerance, compared with its intolerant parent. The tolerant line took up 50% more Zn when grown in Zn-deficient soil, and this coincided with increased expression of putative ligand-efflux genes in the roots. The lines secreted similar amounts of DMA under Fe deficiency. Using the same tolerant and intolerant rice lines, Arnold *et al.* (2010) found evidence supporting the involvement of PS secretion in Zn uptake, using stable isotope fractionation. They found a preference for heavy ⁶⁶Zn in the tolerant rice line grown in soil, but not in the intolerant, suggesting the involvement of a Zn-binding process and uptake of complexed Zn. By contrast, rice grown in nutrient culture showed a light Zn bias, consistent with kinetic fractionation during membrane transport (Weiss *et al.* 2005). A similar heavy isotope fractionation was observed by Guelke & von Blanckenburg (2007) in 'strategy II' type grasses that rely on the PS-Fe uptake pathway for their Fe uptake. This observation and the greater DMA secretion found by Widodo *et al.* (2010) suggest that tolerance to low Zn is, at least in part, because of enhanced DMA secretion and uptake of DMA-Zn complexes.

However, compared with other grasses, rice secretes only small quantities of PS (Takagi 1993; Suzuki *et al.* 2008). The rates of DMA secretion per unit root mass found by Widodo *et al.* (2010) are an order of magnitude smaller than

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those reported for low-Fe tolerant wheat or barley. Further, there is conflicting evidence for enhanced PS secretion in response to Zn stress in rice (Suzuki *et al.* 2008; Widodo *et al.* 2010) and in grasses in general (Walter *et al.* 1994; Pedler, Parker & Crowley 2000; Roberts *et al.* 2004; Suzuki *et al.* 2006). However, rice may be a special case because of the peculiar biogeochemistry of submerged soils, which results not only in widespread Zn deficiency, as discussed previously, but also rare Fe deficiency, which is the main driver of PS secretion in other grasses. The order of binding strengths of DMA and other PS with Zn and Fe species is $\text{Fe(II)} < \text{Zn(II)} < \text{Fe(III)}$ (Murakami *et al.* 1989). So the absence of Fe(III) in submerged soil – at least in the soil bulk – may enhance the effectiveness of PS in complexing and solubilizing Zn.

The question arises: are the observed rates of PS secretion in rice sufficient to solubilize significant amounts of Zn and significantly affect uptake? To answer this requires a quantitative understanding of the processes controlling PS secretion, its transport away from the root, its decomposition by soil microbes, its simultaneous reaction with the soil solubilizing Zn, subsequent transport of the solubilized Zn towards the root and uptake of the PS-Zn complex at the root surface. Arnold *et al.* (2010) made a preliminary model of these processes with which to assess the extent to which observed DMA secretion in rice can explain observed Zn uptake. The model made simplistic assumptions about the dynamics of secretion, the dynamics of root growth, inter-root competition effects, the soil chemistry of DMA and Zn, and DMA decomposition. In this paper, we develop Arnold *et al.*'s model to allow more realistically for these processes.

In particular, we explore the effects of root growth dynamics and localization of secretion in the region of the root tip, and diurnal variation in secretion. A feature of PS secretion is that it appears to be diurnally regulated with a maximum 2–3 h after sunrise, whereas PS production in the roots appears to have no diurnal control (Takagi, Nomoto & Takemoto 1984; Nagasaka *et al.* 2009). This contrasts with other major root secretions (e.g. proteinaceous amino acids, organic acids, sugars; Jones *et al.* 2009) and suggests that the diurnal PS pattern has a functional significance to increase the effectiveness of secretion, possibly linked to non-linear sorption of PS in the soil, rates of decomposition by soil microbes or transport effects (Takagi, Kamei & Yu 1988; Römheld 1991; Reichman & Parker 2007). We explore these matters with the model.

We parameterize the model for rice in submerged soil. However, the model and many of the conclusions we draw from it are generic.

DESCRIPTION OF THE MODEL

We consider the influence of a PS Y on the solubility and uptake of a micronutrient metal X. Our model is based on that of Kirk (1999) for solubilization of phosphate by organic anions secreted from roots. It differs from Kirk's model in two respects. Firstly, we consider the secretion to

be localized in the region behind the root tip and allow for three-dimensional diffusion both radially around the root and longitudinally in the direction parallel to the root axis. Secondly, we generalize the treatment of the interactions of X and Y with the soil solid and with each other in a way that is consistent with the conservation equations for transport and reaction.

We consider that X and Y move by diffusion and convection through the soil pore solution with simultaneous adsorption and desorption on soil surfaces, but with no diffusion in the sorbed state. If X_L , Y_L are the concentrations in the soil solution, and X_S , Y_S are the concentrations in the soil solid in rapid equilibrium with the solution, then the conservation equations for X and Y in unit volume of soil are

$$\partial_t(\theta X_L + X_S) = \nabla \cdot (D_X \nabla X_L - v X_L) - g_X \quad (1)$$

$$\partial_t(\theta Y_L + Y_S) = \nabla \cdot (D_Y \nabla Y_L - v Y_L) - g_Y \quad (2)$$

where θ is the soil water content, D_X , D_Y are the diffusion coefficients of X and Y in the soil, v is the water flux to the root, and g_X , g_Y are functions for the removal or addition of X and Y in processes that are slow or comparable with rates of diffusion through the soil (e.g. slow adsorption-desorption reactions, or consumption by soil microbes). The diffusion coefficients are $D_X = D_{LX}\theta f$ and $D_Y = D_{LY}\theta f$, where D_{LX} , D_{LY} are the diffusion coefficients in free solution and f is an impedance factor.

We specify the following simple relations for equilibration between the solid and solution, allowing for the solubilization of X in the solid by Y in solution and vice versa:

$$\partial_t X_S = \beta_1 X_L - \beta_2 X_S - \beta_3 X_S Y_L \quad (3)$$

$$\partial_t Y_S = \gamma_1 Y_L - \gamma_2 Y_S - \gamma_3 Y_S X_L \quad (4)$$

where β_1 , β_2 , β_3 , γ_1 , γ_2 and γ_3 are reaction rate coefficients. (Note that, unlike the corresponding relations in Kirk's model, these equations cannot generate negative values of X_S or Y_S .) At equilibrium, $\partial_t X_S = 0$ and $\partial_t Y_S = 0$. Therefore

$$X_S = \frac{\beta_1 X_L}{\beta_2 + \beta_3 Y_L} \quad (5)$$

$$Y_S = \frac{\gamma_1 Y_L}{\gamma_2 + \gamma_3 X_L} \quad (6)$$

Differentiating Eqns (5) and (6) gives

$$\partial_t X_S = \frac{\beta_1}{\beta_2 + \beta_3 Y_L} \partial_t X_L - \frac{\beta_1 X_L \beta_3}{(\beta_2 + \beta_3 Y_L)^2} \partial_t Y_L \quad (7)$$

$$\partial_t Y_S = \frac{\gamma_1}{\gamma_2 + \gamma_3 X_L} \partial_t Y_L - \frac{\gamma_1 Y_L \gamma_3}{(\gamma_2 + \gamma_3 X_L)^2} \partial_t X_L \quad (8)$$

Combining Eqns (1) and (7), and (2) and (8) gives for the overall conservation equations in terms of X_L and Y_L :

$$\left(\theta + \frac{b_X}{1 + \kappa_X b_X Y_L}\right) \partial_r X_L - \frac{\kappa_X b_X^2 X_L}{(1 + \kappa_X b_X Y_L)^2} \partial_r Y_L \quad (9)$$

$$= \nabla \cdot (D_X \nabla X_L - v X_L) - g_X$$

$$\left(\theta + \frac{b_Y}{1 + \kappa_Y b_Y X_L}\right) \partial_r Y_L - \frac{\kappa_Y b_Y^2 Y_L}{(1 + \kappa_Y b_Y X_L)^2} \partial_r X_L \quad (10)$$

$$= \nabla \cdot (D_Y \nabla Y_L - v Y_L) - g_Y$$

where $b_X = \beta_1/\beta_2$, $b_Y = \gamma_1/\gamma_2$, $\kappa_X = \beta_3/\beta_1$ and $\kappa_Y = \gamma_3/\gamma_1$. The parameters b_X and b_Y are the buffer powers for X and Y at $Y_L = 0$ and $X_L = 0$, respectively: $b_X = (\partial X_S/\partial X_L)_{Y_L=0}$ and $b_Y = (\partial Y_S/\partial Y_L)_{X_L=0}$. κ_X and κ_Y are X–Y and Y–X interaction coefficients. The evaluation of these parameters is discussed under ‘Parameter values’.

Boundary conditions

The geometry of the model is given in Fig. 1. The concentrations X_L and Y_L governed by Eqns (9) and (10) depend on two space variables: radial distance from the root centre r , and longitudinal distance from the base of the root z . We consider a root growing at a constant rate G , such that the location of the root tip at time t is given by $L = L_0 + G \times t$. We further consider that uptake of X occurs in a region δL_X long behind the root tip, and that secretion of Y only occurs in a region δL_Y long just behind the tip. Therefore, the boundary conditions for uptake of X and secretion of Y are

$$D_X \partial_r X_L - v X_L = \alpha X_L \quad \text{at } r = a, L \leq z \leq L - \delta L_X \quad (11)$$

$$D_Y \partial_r Y_L - v Y_L = -F_Y(t) \quad \text{at } r = a, L \leq z \leq L - \delta L_Y \quad (12)$$

where α is the root absorbing power for X, $F_Y(t)$ is the rate of exudation of Y and a is the root radius. Crucially, $F_Y(t)$ is a function of time. We compare two limiting cases: (1) exudation occurs for 2 h in the morning; and (2) exudation is continuous over 24 h. In both cases, the total exudation over 24 h is the same. Hence, if the total exudation is M , then for case (1) $F_Y = M/(2 \times 60 \times 60)$ during exudation and for case (2) $F_Y = M/(24 \times 60 \times 60)$ continuously.

At the boundary between the zones of influence of neighbouring roots, $r = x$, we specify no transfer of X and Y, that is

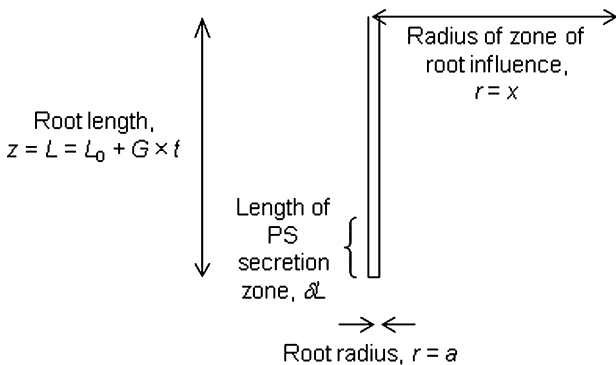


Figure 1. Geometry of the model.

$$D_X \partial_r X_L - v X_L = 0 \quad \text{at } r = x \quad (13)$$

$$D_Y \partial_r Y_L - v Y_L = 0 \quad \text{at } r = x \quad (14)$$

For a parallel array of roots, uniformly or randomly distributed in the soil, $x = 1/\sqrt{\pi L_V}$, where L_V is the root length density.

Ahead and behind the root tip we make the boundary in the z direction sufficiently distant that it does not influence the concentration gradients of X or Y.

Solution of the equations

We solved Eqns (9) and (10) subject to the boundary conditions using the COMSOL Multiphysics 3.5a standard core solver (Cambridge, UK).

Parameter values

We use plant parameter values appropriate for rice and soil parameters appropriate for highly Zn-deficient rice soils and the PS DMA. The parameter values are listed in Table 1. We derived the values as follows.

Parameters for Zn sorption, interaction with DMA and immobilization (b_X , κ_X and g_X)

From Eqn (5) and the definitions of b_X and κ_X :

$$X_S = \left(\frac{b_X}{1 + b_X \kappa_X Y_L}\right) X_L \quad (15)$$

$$\therefore \frac{X_L}{X_S} = \frac{X_L}{X - \theta X_L} = 1/b_X + \kappa_X Y_L$$

Therefore, if an amount of Y is mixed well into a portion of soil and the equilibrium concentrations of X and Y in solution measured, plots of $X_L/(X - \theta X_L)$ against Y_L should have slope κ_X and intercept $1/b_X$.

Arnold *et al.* (2010) estimate a typical value for the soil Zn buffer power of $b_X = 200$.

From the equilibrium stability constants of DMA, and assuming that competition with other cations is unimportant (see next paragraph), the expected concentration of complexed Zn(II) in a solution with DMA is

$$\frac{[\text{ZnDMA}^-]}{[\text{Zn}^{2+}][\text{DMA}_{\text{tot}}]} \quad (16)$$

$$= \frac{K_f}{1 + [\text{H}^+]/K_{a3} + [\text{H}^+]^2/(K_{a2}K_{a3}) + [\text{H}^+]^3/(K_{a1}K_{a2}K_{a3})}$$

where $K_{a1,2,3}$ are the H^+ dissociation constants for the last carboxylate group and two amino groups of DMA, and K_f is the formation constant for ZnDMA^- . $\text{p}K_{a1,2,3} = 3.19, 8.25, 10.00$ and $\text{p}K_f = -12.82$ ($T = 25^\circ\text{C}$, $I = 0.1 \text{ M}$; Murakami *et al.* 1989). Therefore, at $\text{pH} = 7.0$ (the approximate pH of most soils following

Table 1. Model nomenclature and values of parameters used (references in main text)

Symbol	Meaning	Value
θ	Solution volume fraction	$0.7 \text{ dm}^3 \text{ dm}^{-3}$
f	Diffusion impedance factor	0.5
D_{LX}	Diffusion coefficient of Zn species in free solution	$7 \times 10^{-8} \text{ dm}^2 \text{ s}^{-1}$
D_{LY}	Diffusion of coefficient of DMA in free solution	$7 \times 10^{-8} \text{ dm}^2 \text{ s}^{-1}$
b_X	Zn buffer power	200
b_Y	DMA buffer power	Either 1 or $\rho mn/(n + Y_L)^2$, where $m = 1 - 10 \times 10^{-5} \text{ mol kg}^{-1}$ and $n = 10 \times 10^{-6} \text{ M}$
κ_X	Zn–DMA interaction coefficient	$5 \times 10^3 \text{ dm}^3 \text{ mol}^{-1}$
κ_Y	DMA–Zn interaction coefficient	0
g_X	Function for Zn immobilization	0
g_Y	Function for DMA decomposition	$\rho V_{\max} Y_L / (K_M + Y_L)$ where $V_{\max} = 2.5 - 10 \times 10^{-9} \text{ mol kg}^{-1} \text{ s}^{-1}$ and $K_M = 100 \times 10^{-6} \text{ M}$
ρ	Soil bulk density	1 kg dm^{-3}
$X_{L,0}$	Initial Zn concentration in soil solution	$0.01 \times 10^{-6} \text{ M}$
α	Zn absorbing power of root	$1.5 \times 10^{-3} \text{ dm s}^{-1}$
F_Y	Rate of DMA exudation over 24 h	$4 \times 10^{-11} \text{ mol dm}^{-2} \text{ s}^{-1}$
v	Water flux	$1 \times 10^{-6} \text{ dm s}^{-1}$
a	Root radius	$1 \times 10^{-3} \text{ dm}$
L	Root length	increases at $G = 0.2 \text{ dm d}^{-1}$
δL_X	Length of Zn uptake zone	L
δL_Y	Length of PS secretion zone	0.2 dm
L_V	Maximum root length density	100 to 4,000 dm dm^{-3}
x	Radius of zone of root influence	$1/\sqrt{\pi L_V}$

Component X is Zn and component Y is the phytosiderophore deoxymugineic acid (DMA). PS, phytosiderophore; Zn, zinc.

submergence), $[ZnDMA^-]/[DMA_{\text{tot}}] \approx X_L/Y_L = 3.5 \times 10^7 \times [Zn^{2+}]$. From Eqn (15), if $1/b_X < \kappa_X Y_L$ and $\theta X_L \ll X$, then $\kappa_X = X_L/Y_L \cdot 1/X$. Therefore, for $[Zn^{2+}]/X \approx 10^{-4}$, which is realistic, $\kappa_X \approx 5 \times 10^3 \text{ dm}^3 \text{ mol}^{-1}$.

There is the possibility of competition of Zn^{2+} for DMA with other cations, in which case the effective value of κ_X would be smaller and a more complex model would be required. We have ignored this for this stage of model development. However, from the following considerations, such effects are likely to be small. The order of conditional stability constants for the binding of relevant cations by DMA at pH 7.0 (similar to those in Eqn 16, but also allowing for the hydrolytic nature of Fe^{3+}) is $Cu^{2+} > Fe^{3+} > Ni^{2+} > Zn^{2+} > Fe^{2+} > Mn^{2+}$ (Murakami *et al.* 1989). In submerged rice soils, Fe species will generally have by far the greatest concentrations out of these cations. The conditional stability constant of Fe^{2+} –DMA species is two orders of magnitude smaller than that of Zn^{2+} , so competition with Fe^{2+} is unlikely to be important. The conditional stability constant of Fe^{3+} –DMA species is much larger. Some Fe(III) oxide is formed in the rice rhizosphere as a result of Fe(II) oxidation by O_2 released from the root (see Discussion). However, the zone of Fe(III) oxide formation is close to the root surface, whereas Zn solubilization by DMA occurs over a much greater distance from the root, where there will be no competition with Fe^{3+} .

We assume there are no slow reactions involving Zn on the time scale of diffusion in the soil, so $g_X = 0$.

Parameters for DMA sorption, interaction with Zn and decomposition (b_Y , κ_Y and g_Y)

Hiradate & Inoue (2000) measured mugineic acid sorption by a wide range of soils and found that, although > 50% of added DMA was sorbed in soils with pH < 6.5, it was largely non-adsorbed in soils with pH > 7.5, and in neutral soils the median amount sorbed was 5% (at soil : solution ratio 1:25). This indicates that for DMA sorption in many submerged soils, $b_Y \approx 1 - 10$. To explore the effect of non-linearity in DMA sorption (and resulting non-linear interactions with a diurnally varying secretion of DMA), we used a Langmuir-type sorption curve:

$$Y_s = \frac{\rho m Y_L}{n + Y_L}$$

$$\therefore b_Y = \left(\frac{\partial Y_s}{\partial Y_L} \right)_{X_L=0} = \frac{\rho m n Y_L}{(n + Y_L)^2} \quad (17)$$

where m and n are coefficients. From the known behaviour of DMA-like compounds in soils (Oburger *et al.* 2009), appropriate values are $m = 1 - 10 \times 10^{-5} \text{ mol kg}^{-1}$ and $n = 10 \times 10^{-6} \text{ M}$.

Because the amount of DMA reacting with the soil is much larger than the amount of Zn being removed by the plants, the diffusion of Zn will have little influence on the diffusion of DMA, so $\kappa_Y = 0$.

For the function g_Y for the rate of decomposition of DMA, we take as the simplest case linear dependence on

the concentration in solution. To explore the effect of non-linearity, we consider a Michaelis–Menten-type dependence on the concentration in solution:

$$g_Y = \frac{\rho V_{\max} Y_L}{K_M + Y_L} \quad (18)$$

where V_{\max} and K_M are coefficients. From known rates of persistence of DMA in soils (Takagi *et al.* 1988), appropriate values are $V_{\max} = 2.5\text{--}10 \times 10^{-9} \text{ mol kg}^{-1} \text{ s}^{-1}$ and $K_M = 100 \times 10^{-6} \text{ M}$.

Parameters for root growth and Zn uptake (α , F_Y , a , δL_X , δL_Y , G and L_V)

Rates of DMA release from seedling roots in solution culture systems are 100–500 pmol g⁻¹ root FW s⁻¹ during the 2 h secretion period in Fe-deficient barley and wheat (Tolay *et al.* 2001; Suzuki *et al.* 2006; Reichman & Parker 2007), but are at least an order of magnitude smaller than this (i.e. approx. 10 pmol g⁻¹ root FW s⁻¹) in rice (Takagi 1993; Suzuki *et al.* 2008; Widodo *et al.* 2010). For an average root radius $a = 0.01 \text{ cm}$, 10 pmol g⁻¹ root FW s⁻¹ is equivalent to $F_Y = 0.05 \text{ pmol cm}^{-2} \text{ s}^{-1}$.

We assign the root Zn absorbing power a sufficiently large value that uptake is insensitive to it: $\alpha = 1.5 \times 10^{-2} \text{ cm s}^{-1}$. We take for the lengths of the zones of Zn uptake and DMA secretion $\delta L_X = \text{whole root length}$ and $\delta L_Y = 0.2 \text{ dm}$.

Realistic values for the root geometry parameters for rice in flooded soil are root radius $a = 0.001 \text{ dm}$, initial root length $L_0 = 0.5 \text{ dm}$, rate of root elongation $G = 0.2 \text{ dm d}^{-1}$ and root length density $L_V = 50 \text{ to } 5,000 \text{ dm dm}^{-3}$ (Morita & Yamazaki 1993).

Water flux (v)

The flux of water in the transpiration stream – and its diurnal variation relative to PS secretion – might affect the spread of PS away from the root and the return of solubilized Zn towards it. However, the following calculations show that convection of PS and Zn in this way will not be important.

We consider the relevant equations in dimensionless form (Roose & Kirk 2009). The flux, $F_Y(t)$, of PS across the root is given by Eqn (12). The dimensionless form of this equation is

$$\partial_R Y - PeY = -\frac{F_Y(t)a}{D_Y[Y]} \quad R=1 \quad (19)$$

where $R = r/a$, $Y = [Y]/Y_L =$ and $Pe = av/D_Y$. The dimensionless variable Pe is the Peclet number. The relevant scale for the PS concentration is $[Y] = F_{Y\max}a/D_Y$, where $F_{Y\max}$ is the maximum flux of PS from the root. Hence the dimensionless boundary condition is

$$\partial_R Y - PeY = -F(t) \quad R=1 \quad (20)$$

where $F(t)$ is the dimensionless flux, whose value is between 0 and 1. It follows that if $Pe \ll 1$, the effect of convection will be negligible.

With $v = 10^{-6} \text{ dm s}^{-1}$, which corresponds to fast transpiration (Tinker & Nye 2000), and the other parameter values as in Table 1, $Pe = 4 \times 10^{-2}$, so convection will be unimportant compared with diffusion. Similar considerations apply to the movement of solubilized Zn towards the root. We conclude that transpiration will not significantly affect Zn solubilization and uptake by PS in rice. We therefore ignore it in our subsequent calculations. Note that these conclusions are for submerged soils, where diffusion coefficients are relatively large and therefore Peclet numbers are small.

RESULTS AND DISCUSSION

Calculated concentration-distance profiles

Figure 2 shows the calculated profiles of PS in the soil solution near the root over time with a diurnally varying secretion, and the corresponding profiles of Zn in the soil solution and changes in Zn in the whole soil. It shows that the concentration of PS in solution is greatest close to the root surface in the secretion zone and decreases with distance away. A trace of the previous day's secretion is evident in the region behind the secretion zone. However, as a result of decomposition by microbes, little PS persists longer than a day. Because the length of the secretion zone (2 cm) is comparable with the distance the root grows in a day, secretion is largely into fresh soil at all times, and lateral diffusion from the previous day's secretion makes little difference to this.

By contrast, the calculated profiles of Zn in the soil solution (middle panels in Fig. 2) show depletion of Zn close to the root surface and a peak some distance away where Zn has been solubilized by PS. The PS-Zn complex diffuses away from this region, both towards the root and away from it. After 3 d, some of the Zn solubilized has spread as far as the edge of the zone of root influence ($r = x$). It then influences the concentration profile around the neighbouring root, and therefore increases the net recovery of solubilized Zn by the root system. This effect will increase as x decreases (i.e. greater rooting density).

The bottom panels in Fig. 2 give the calculated changes in Zn concentration in the whole soil (i.e. the change in solution plus solid Zn from the initial value). It shows that, as a result of PS secretion, the plant is able to extract Zn from a much wider region than it otherwise would.

Calculated cumulative Zn uptake

Figure 3 shows the calculated sensitivity of cumulative Zn uptake by the root to diurnal variation in PS secretion. It compares 2 h secretion with the same net secretion spread over 24 h to see if the diurnal cycle results in any net increase in uptake over time. It appears that it does not. Though the effect of diurnal variation is evident on the day

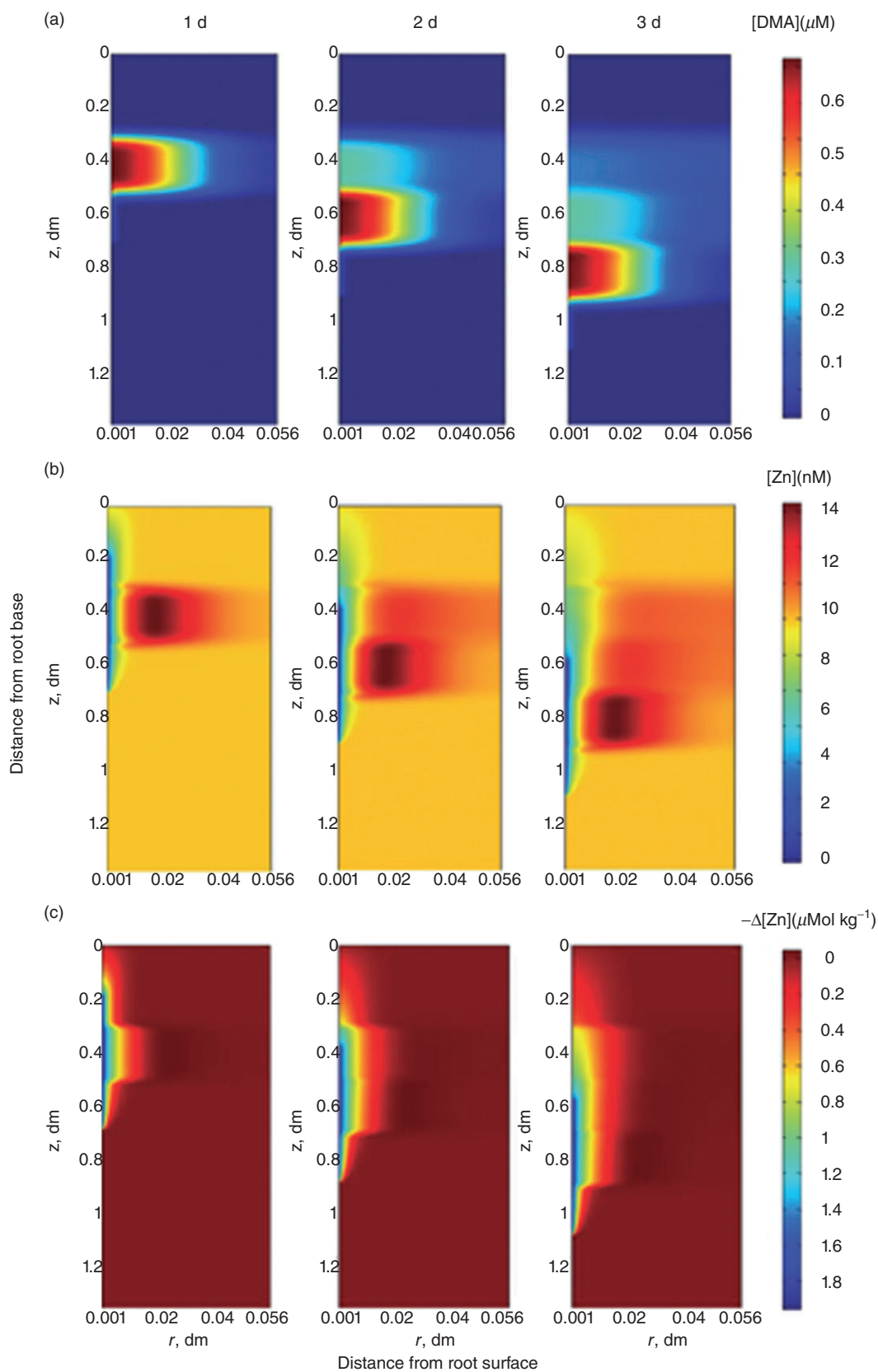


Figure 2. Calculated concentration-distance profiles over time near a growing root secreting a phytosiderophore (DMA) and absorbing Zn: (a) DMA in the soil solution; (b) Zn in the soil solution; (c) changes in Zn in the whole soil. Secretion and uptake are confined to 2 cm behind the root tip. Secretion occurs for 2 h d^{-1} . Root grows at 2 cm d^{-1} . DMA, deoxymugineic acid; Zn, zinc.

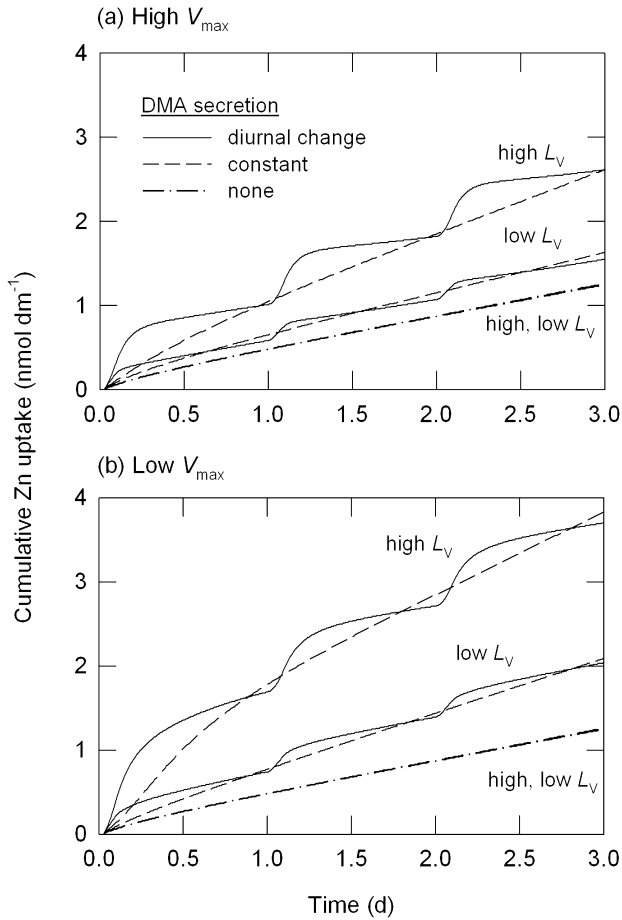


Figure 3. Calculated cumulative Zn uptake per unit whole-root length over time for different values of root length density (high, low $L_v = 4,000, 1,000 \text{ dm dm}^{-3}$) with or without DMA secretion (constant or diurnally changing) with (a) high decomposition rate ($V_{\max} = 25 \text{ nmol dm}^{-3} \text{ s}^{-1}$) and (b) low decomposition rate ($V_{\max} = 2.5 \text{ nmol dm}^{-3} \text{ s}^{-1}$). DMA, deoxymugineic acid; Zn, zinc.

of secretion, cumulative uptake over a few days is little altered, irrespective of rooting density and the rate of PS decomposition in the soil.

Figure 4 shows the same comparison of varying secretion but with a non-linear relationship for PS sorption on the soil. In principle, with the non-linear sorption relation in Eqn (17), a pulsed secretion may result in a greater concentration of PS in the soil solution and therefore greater Zn solubilization. But it appears that it does not. While non-linear sorption does qualitatively influence the cumulative Zn uptake, quantitatively the results are almost indistinguishable within likely experimental errors. Thus, we conclude that non-linear sorption of PS does not play a significant role in Zn acquisition.

The rate of PS consumption by microbes, as indicated by varying V_{\max} , does influence cumulative Zn uptake. However, an order of magnitude decrease in V_{\max} produces only a 16% increase in uptake over 3 d.

Figure 5 shows the calculated effect of root growth into fresh soil compared with a static root. The static

root exhausts the available Zn in the soil around it faster than the root exploring fresh soil, and over time the cumulative uptake is less. Further, the effect of diurnal variation in DMA secretion is dampened with a static root.

The parameter that most influences cumulative uptake according to our sensitivity analysis is root length density, L_v . The effect of PS secretion in Figs 3 and 4 is much larger at large values of L_v . The explanation is that with larger L_v , the zones of influence of neighbouring roots overlap faster (Fig. 2), whereupon neighbouring roots benefit from Zn solubilized by each other. This phenomenon explains the finding of Hoffland *et al.* (2006) that genotype differences in Zn uptake per plant by rice in Zn-deficient submerged soil diminish with increasing planting density. Because diffusion rates are much greater in submerged soil than in moist soil, other factors being equal, the effect of rooting density and in general the recovery of solubilized Zn by the plant per unit PS secreted are greater. Hence, rice plants in submerged soil may increase their Zn uptake with far smaller rates of PS secretion.

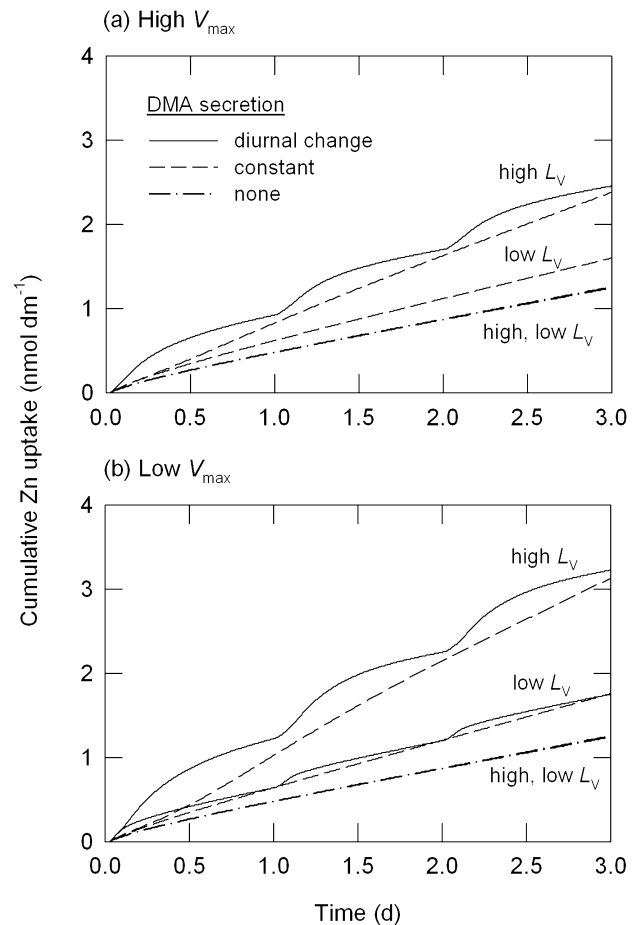


Figure 4. As Fig. 3 but with non-linear PS buffering by the soil (Eqn 17). DMA, deoxymugineic acid; PS, phyto siderophore; Zn, zinc.

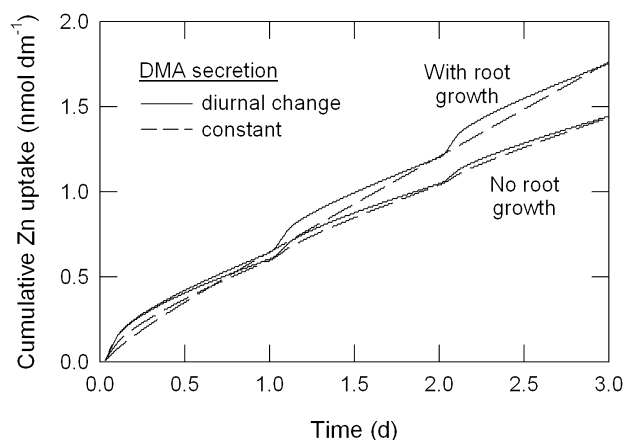


Figure 5. The effect of root growth into fresh soil versus no root growth. Parameter values as in Figs 3 and 4 with $L_V = 100 \text{ dm dm}^{-3}$, $V_{\max} = 2.5 \text{ nmol dm}^{-3} \text{ s}^{-1}$ and non-linear PS buffering by the soil. DMA, deoxymugineic acid; PS, phytosiderophore; Zn, zinc.

Other considerations

There are several potential complications in the rice rhizosphere not considered in our model. Oxidation of Fe(II) by O_2 released from the roots will deposit ferric hydroxide on and near root surfaces. Simultaneously, H^+ is formed in the oxidation reaction



tending to lower the rhizosphere pH (Begg *et al.* 1994; Kirk & Bajita 1995). Further H^+ is released by rice roots to balance excess intake of cations over anions, the main form of N taken up being NH_4^+ . The resulting pH change will depend on buffering by the soil and movement of H^+ away by acid-base transfer, principally by diffusion of HCO_3^- towards the acidification zone and reaction with H^+ to form H_2CO_3 and CO_2 . This is generally fast in submerged soil because of the high moisture content, near neutral pH and high CO_2 pressure, which together mean that concentrations of HCO_3^- are high. Calculations and measurements reviewed by Kirk (2004) indicate that pH changes of 0.2–0.5 units at the root surface are likely to be typical.

Will these effects alter Zn solubility and uptake? Acidification will solubilize soil Zn, but some Zn will be re-sorbed on the freshly formed ferric hydroxide close to the root (Kirk & Bajita 1995). Also, a decrease in pH will decrease the effectiveness of DMA in complexing Zn (Eqn 16), as will the presence of Fe(III), especially at low pH. Nonetheless, the results of Arnold *et al.* (2010) on isotope fractionation during Zn uptake (Introduction) provide strong evidence that the bulk of uptake is via complexation with DMA. Arnold *et al.* (2010) show that the heavy Zn isotope fractionation they observed in rice grown in submerged soil could only be explained by complexation of soil Zn with a complexing agent and uptake of the complexed Zn. A tentative explanation of the said observations

is that Zn is solubilized by reaction of H^+ with the soil and the solubilized Zn is kept in solution by complexation with DMA. Reichard, Kretzschmar & Kraemer (2007) discuss such proton- and ligand-promoted dissolution reactions for Fe(III) solubilization by siderophores. From the stoichiometry of Eqn (21), and adding the flux of H^+ because of the root cation–anion balance, the rate of formation of H^+ is typically four or five times that of Fe(III) (Begg *et al.* 1994; Kirk & Bajita 1995), though it causes only small changes in pH. So solubilization by acidification will dominate the immobilization and decreased complexation effects. In terms of our model, this is equivalent to maintaining a constant value of the DMA–Zn interaction coefficient, κ_Y , as solubilizable Zn is removed from the soil.

CONCLUSIONS

- 1 Observed rates of DMA secretion from rice are sufficient to account for observed rates of Zn uptake, given realistic estimates of the Zn-solubilizing effect of DMA in submerged soil.
- 2 There is a strong interaction between DMA secretion and rooting density in rice, as a result of which neighbouring roots benefit from DMA secreted by each other. This can greatly increase the recovery of Zn by a root per unit DMA secretion.
- 3 Convection in the transpiration stream does not greatly diminish the spread of DMA away from the root, nor increase the return of solubilized Zn towards the root.
- 4 Because the length of the secretion zone is comparable with the distance the root grows in a day, secretion is largely into fresh soil at all times, and lateral diffusion from the previous day's secretion makes little difference to this because little DMA persists more than a day.
- 5 Diurnal variation in secretion has little effect on cumulative Zn uptake per unit daily Zn secretion, indicating that the observed diurnal variation is because of some internal plant mechanism, and is not causally related to Zn uptake efficiency.
- 6 Other complications of rice rhizosphere chemistry do not greatly affect the mentioned conclusions.

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