Soil carbon dioxide venting through rice roots

Guy J.D. Kirk1 | Andrea Boghi1,2 | Marie-Cecile Affholder1 | Samuel D. Keyes2 | James Heppell2 | Tiina Roose2

1 School of Water, Energy and Environment, Cranfield University, Cranfield, UK
2 Faculty of Engineering and Environment, University of Southampton, Southampton, UK

Correspondence
G. Kirk, School of Water, Energy and Environment, Cranfield University, Cranfield MK43 0AL, UK.
Email: g.kirk@cranfield.ac.uk

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Abstract
The growth of rice in submerged soils depends on its ability to form continuous gas channels—aerenchyma—through which oxygen (O2) diffuses from the shoots to aerate the roots. Less well understood is the extent to which aerenchyma permits venting of respiratory carbon dioxide (CO2) in the opposite direction. Large, potentially toxic concentrations of dissolved CO2 develop in submerged rice soils. We show using X-ray computed tomography and image-based mathematical modelling that CO2 venting through rice roots is far greater than thought hitherto. We found rates of venting equivalent to a third of the daily CO2 fixation in photosynthesis. Without this venting through the roots, the concentrations of CO2 and associated bicarbonate (HCO3−) in root cells would have been well above levels known to be toxic to roots. Removal of CO2 and hence carbonic acid (H2CO3) from the soil was sufficient to increase the pH in the rhizosphere close to the roots by 0.7 units, which is sufficient to solubilize or immobilize various nutrients and toxicants. A sensitivity analysis of the model showed that such changes are expected for a wide range of plant and soil conditions.

KEYWORDS
biological models, biological transport, X-ray computed tomography

1 INTRODUCTION

Large dissolved CO2 concentrations develop in submerged rice soils (equivalent partial pressures 5–70 kPa—Greenway, Armstrong, & Colmer, 2006; Kirk, 2004; Ponnamperuma, 1972) because CO2 formed in root and soil respiration escapes only slowly by diffusion through the water-filled soil pores. Carbon dioxide is produced in anaerobic respiration in the soil bulk and in aerobic respiration in the rhizosphere fuelled by O2 and organic substrates released from the roots (Figure 1). There is therefore a large CO2 gradient between the soil and the aerenchyma inside the root. Hence, CO2 will enter the roots by diffusion and mass flow in the transpiration stream and be vented to the shoots and atmosphere by diffusion through the aerenchyma (Higuchi, Yoda, & Tensho, 1984). There has been much research on this pathway for CH4 emission from ricefields (Butterbach-Bahl, Papen, & Rennenberg, 1997; Nouchi, Mariko, & Aoki, 1990; Schütz, Seiler, & Conrad, 1989; Wang, Akiyama, Yagi, & Yan, 2018), but CO2—which is >20 times less volatile than CH4—has received little attention. High CO2 concentrations and associated HCO3− can be toxic to root cells, and therefore, some degree of venting is necessary for healthy growth (Greenway et al., 2006). Also, removal of dissolved CO2 will tend to increase the pH of the rhizosphere soil, with consequences for the ricefield biogeochemistry (Affholder, Weiss, Wissuwa, Johnson-Beebout, & Kirk, 2017; Begg,
FIGURE 1  Gas formation and venting through rice roots in paddy soil. (a) Cross section showing roots and water-saturated, anaerobic soil. (b) Root aerenchyma. (c) Cut-away X-ray computed tomography image of roots (green) and soil gas bubbles (white). (d) Gas generating and consuming processes in the soil (after inorganic oxidants have been exhausted): (1) aerobic decomposition of soil organic matter (SOM) in the rhizosphere, (2) anaerobic decomposition of SOM in the soil bulk (a–d are coefficients), (3) CH₄ production from acetate, (4) CH₄ production from H₂, (5) CH₄ oxidation, and (6) Fe (II) oxidation. Gas bubbles become entrapped under soil particles, but there is no continuous gas phase through the soil [Colour figure can be viewed at wileyonlinelibrary.com].

Kirk, MacKenzie, & Neue, 1994; Kirk & Bajita, 1995). Two further processes affect the chemistry of the rice rhizosphere: oxidation of inorganic reductants, such as ferrous iron, by O₂ from the roots and associated generation of H⁺, and release of H⁺ from the roots to balance excess intake of cations (particularly NH₄⁺) over anions (Kirk, 2004). These inputs of H⁺ will tend to offset H⁺ consumption in venting of dissolved CO₂ from the soil and the resulting changes in carbonate equilibria.

Investigating such processes is challenging given the sensitivity of gas fluxes to measurement conditions. A key problem is how to separate the fluxes of soil-derived CO₂ from those of root- and shoot-derived CO₂. This might be done, for example, with isotopically labelled carbon sources, if it were possible to ensure uniform labelling and complete separation of the plant and soil sources. In this study, we avoided these difficulties by directly imaging and quantifying profiles of gas depletion around rice roots growing in submerged soil using X-ray computed tomography (CT) and mathematical modelling.

In brief, we grew initially 4-week-old rice seedlings in a submerged, anaerobic rice soil contained in glass pots, and, after 4 weeks, scanned the pots using X-ray CT imaging to measure the spatial distribution of roots and gas bubbles entrapped in the soil (Figure 1c). The image analysis showed prominent and abundant gas bubbles in the soil bulk, but no or very few bubbles in the soil close to roots, and there was a clear relation between the absence of gas bubbles and high root density, as well as an increasing concentration of bubbles with depth through the soil. Analysis of the bubbles showed they were approximately 40% CO₂ by volume and 60% CH₄. We developed a mathematical model to account for these observations on the basis of the following picture of events.

If the soil solution becomes supersaturated with CO₂ or CH₄, or other volatile products of respiration, gas bubbles will form and tend to become entrapped beneath soil particles. If the bubbles become sufficiently large, or if the soil is agitated by some mechanical disturbance, then the bubbles will rise to the surface by “ebullition.” At steady state (which is typically reached within a few weeks of the soil being submerged—Kirk, 2004; Ponnamparapu, 1972), the volume of bubbles and their composition, as well as the concentrations of dissolved gases in equilibrium with them, will depend on the rates of production versus loss by ebullition and diffusion and venting through the roots. We fitted the model, on the basis of this outline, to the X-ray CT images of roots and gas bubbles. Thereby, we obtained values of the model parameters and the proportions of CO₂ and CH₄ generated in and leaving the soil via the various pathways. The details follow.

2 | MATERIALS AND METHODS

2.1 | Model development

We describe the steady-state transport of each dissolved gas through the soil by the following continuity equation:

\[ \nabla \cdot D_i \nabla C_{iU} - \nu C_{iU} + S_i - E_i - R_i = 0, \]

where \( C_{iU} \) is the concentration of dissolved gas \( i \), \( D_i \) is its diffusion coefficient through the soil solution, \( \nu \) is the water flux into roots, \( S_i \) is the rate of gas production, \( E_i \) is the rate of ebullition, and \( R_i \) is the rate of root-mediated efflux. There is an equation of this form each for dissolved CO₂, CH₄, and N₂, which enters the soil by diffusion from the atmosphere and roots. For CO₂, \( C_{iU} \) is adjusted for the concentration of dissolved CO₂ plus the concentration of HCO₃⁻ in equilibrium with it (CO₃²⁻ is unimportant at the near neutral pH of most submerged soils).

In Equation (1), the diffusion coefficient, \( D_i = D_i \theta L \), where \( D_i \) is the diffusion coefficient in free solution, \( \theta L \) is the soil volumetric water content, and \( f_L \) is a tortuosity factor (Kirk, 2004). The volumetric gas content, \( \theta_G \) (from which \( \theta_L = \theta - \theta_G \) where \( \theta \) is the total porosity) is proportional to the sum of the partial pressures of the volatile solutes, \( \sum P_i = P_{CO_2} + P_{CH_4} + P_{N_2} + P_{H_2O} \) (\( P_{H_2O} \) is the saturating pressure of H₂O):

\[ \theta_G = K_A \sum P_i, \]
where $K_s$ is a constant that is characteristic of the submerged, paddy soil. From the gas law: $P_i = RTC_i$, where $C_i$ is the concentration of gas $i$ in the soil gases. From Henry’s law: $C_i = C_{GI}/K_{iH}$ where $K_{iH}$ is the dimensionless Henry’s law constant for gas $i$.

We specify the following relations for $S$, $E$, and $R_i$. For $S$, at steady state, CO$_2$ production from soil carbon is constant with depth and time, equal to $S_{CO_20}$, and production from root-derived carbon is proportional to the root length density, $L_V$ (root length per unit soil volume), that is,

$$S_{CO_2} = S_{CO_20} + k_V L_V.$$  

(3)

where $k_V$ is a proportionality constant. At steady state, the ratio of CH$_4$ production to CO$_2$ production is also constant (Kirk, 2004):

$$S_{CH_4} = \alpha_{CH_4} S_{CO_2}.$$  

(4)

For $E_i$, the rate of ebullition is a function of the volume of the gas bubbles: As bubbles grow, they become more buoyant and so are more easily displaced. Hence, taking total gas volume to represent bubble volume:

$$E_i = k_E \theta C_{Gi}.$$  

(5)

where $k_E$ is a rate constant that depends on the physical properties of the soil. For $R_i$, root-mediated efflux from the soil occurs by degassing of dissolved CO$_2$ and CH$_4$ into the root aerenchyma and diffusion through the aerenchyma to the atmosphere (Beckett, Armstrong, Justin, & Armstrong, 1988). We represent this as

$$R_i = k_R L V D_{Gi}(C_i - C_{Gi}),$$  

(6)

where $k_R$ is a root gas transmissivity, $D_{Gi}$ is the diffusion coefficient of gas $i$ in air, $C_i$ is the gas concentration along the profile, and $C_{Gi}$ is the gas concentration at $z = 0$. The root gas transmissivity accounts for all factors limiting CO$_2$, transfer from the soil solution at the root surface to the aerenchyma at the base of the roots at $z = 0$, including the gas permeability of the root wall and epidermis, and the root porosity.

We solved Equations (1)–(6) subject to $C_i$, being constant at the soil–floodwater boundary and there being no flux of gases across the lower boundary. We fitted the model to the observed profiles of gas content by optimizing the values of $k_V$, $k_E$, and $k_R$; all the other parameters were derived independently, and a single set of values was fitted for all replicates and both planting densities (Section 2.4 and Table 1).

### 2.2 Experimental methods

We used the same soil, rice genotype, and growth conditions as in Affholder et al. (2017). In brief, 4-week-old rice seedlings, grown in nutrient culture, were transplanted into pots of submerged, anaerobic rice soil at either one or four plants per pot planted closely together.

#### TABLE 1 Standard parameter values

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Standard value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L$</td>
<td>Soil depth</td>
<td>1.7 dm</td>
<td>Set by experimental conditions</td>
</tr>
<tr>
<td>$\theta$</td>
<td>Soil porosity</td>
<td>0.69</td>
<td>Measured</td>
</tr>
<tr>
<td>$f_L$</td>
<td>Soil liquid diffusion impedance factor</td>
<td>0.35</td>
<td>Based on Kirk, Solivas, &amp; Alberto (2003) for similar soils</td>
</tr>
<tr>
<td>$v$</td>
<td>Water flux into roots</td>
<td>0 dm s$^{-1}$</td>
<td>At $v = 10^{-7}$ dm s$^{-1}$, which is a typical value (Kirk, 2004), the additional CO$_2$ flux into the roots ($=vC_l$) is &lt;2% greater. We therefore use $v = 0$ for simplicity.</td>
</tr>
<tr>
<td>pH</td>
<td>Soil pH</td>
<td>7.0</td>
<td>Measured</td>
</tr>
<tr>
<td>$[H_2CO_3]_0 + [HCO_3^-]_0$</td>
<td>H$_2$CO$_3$ + HCO$_3^-$ concentration at $z = 0$</td>
<td>1 mM</td>
<td>Measured</td>
</tr>
<tr>
<td>$[H_2CO_3]_0 + [HCO_3^-]_i$</td>
<td>H$_2$CO$_3$ + HCO$_3^-$ concentration in bulk soil at $t = 0$</td>
<td>40 mM</td>
<td>Measured</td>
</tr>
<tr>
<td>$[CH_4]_0$</td>
<td>Dissolved CH$_4$ concentration at $z = 0$</td>
<td>2.9 nM</td>
<td>From atmospheric $P_{CH_4}$</td>
</tr>
<tr>
<td>$[N_2]_0$</td>
<td>Dissolved N$_2$ concentration at $z = 0$</td>
<td>0.5 mM</td>
<td>From atmospheric $P_{N_2}$</td>
</tr>
<tr>
<td>$S_{CO_20}$</td>
<td>CO$_2$ production from soil C</td>
<td>9.5 $\times$ 10$^{-6}$ mol dm$^{-3}$ s$^{-1}$</td>
<td>Fitted for unplanted soil, such that $C_l$ in the absence of roots (i.e., $k_V = 0$, $k_T = 0$) agrees with measured value</td>
</tr>
<tr>
<td>$\alpha_{CH_4}$</td>
<td>$S_{CH_4}/S_{CO_2}$</td>
<td>1.0</td>
<td>Set such that $P_{CH_4} \approx P_{CO_2}$</td>
</tr>
<tr>
<td>$K_v$</td>
<td>Equation (2)</td>
<td>2.3 $\times$ 10$^{-2}$</td>
<td>Fitted for unplanted soil</td>
</tr>
<tr>
<td>$k_E$</td>
<td>Rate constant for ebullition</td>
<td>1.0 $\times$ 10$^{-4}$ s$^{-1}$</td>
<td>Fitted</td>
</tr>
<tr>
<td>$k_T$</td>
<td>Root gas transmissivity</td>
<td>9.5 $\times$ 10$^{-4}$</td>
<td>Fitted</td>
</tr>
<tr>
<td>$k_V$</td>
<td>Constant for decomposition of root-derived C</td>
<td>9.3 $\times$ 10$^{-13}$ mol dm$^{-1}$ s$^{-1}$</td>
<td>Fitted</td>
</tr>
</tbody>
</table>
After 4 weeks, the pots were scanned using X-ray CT imaging to measure the spatial distribution of roots and gas bubbles entrapped in the soil (Section 2.3).

The soil was from ricefields at Tiaong, Quezon Province, Philippines. It is a Hydquent (USDA Soil Taxonomy). Portions of topsoil (0- to 30-cm depth) were air dried and sieved to pass <2 mm. The properties of the sieved soil were 42% clay, 40% silt, pH (aerobic in H₂O) 8.5, CEC 9.0 cmolc kg⁻¹, organic carbon content 73 g kg⁻¹, and carbonate content 96 g kg⁻¹ (Izquierdo, Impa, Johnson-Beebout, Weiss, & Kirk, 2016).

Portions (1.2 kg) of the air-dried soil were mixed with 10 g kg⁻¹ of rice straw to stimulate anaerobic reduction processes and then saturated with deionized water and puddled to make a slurry. The slurry was poured into 10-cm-internal-diameter, 21-cm-deep, cylindrical, thin-walled (3-mm) Perspex pots to a depth of 17 cm. The resulting soil bulk density was 0.81 kg dm⁻³, and the volumetric water content was 0.69. The filled pots were inserted into 12-cm-diameter, 21-cm-deep glass pots, and the space between the inner and outer pots were filled with further slurry. This arrangement ensured anoxic conditions in the soil in the inner pot, whereas the thin Perspex wall of the pot was completely transparent to X-rays for imaging after removal from the outer pot. Further deionized water was added to bring the level to the top of the pots, and the water standing in the pots was maintained at this level through the experiment. The soil was allowed to become reduced for 4 weeks at 30°C before transplanting the rice seedlings.

Rice seeds (CV IR55179) were germinated in petri dishes at 30°C in complete darkness for 3 days. The germinated seeds were transferred to a mesh floating on Zn-free Yoshida nutrient solution (Yoshida, Forno, Cook, & Gomez, 1976) and grown for 4 weeks before being transplanted manually into the pre reduced soil in pots. The seedlings were placed with the root crown at approximately 5 cm below the soil-floodwater boundary, as is the practice for growing rice in this soil in the field because of its loose structure and hence weak support for seedlings (Mori et al., 2016). The growth conditions—both before and after transplanting—were 13.5-hr light (600-μmol·m⁻²·s⁻¹ white light) at 30°C and 10.5-hr dark at 24°C.

At 4 weeks after transplanting, the inner Perspex pots were removed and the roots and soil in the pots were imaged as described below. The imaging was complete within 24 hr. The aerial plant parts were then separated from roots at the root crown limit. The fresh biomass was measured, and tillers and leaf number were counted. They were then thoroughly washed with UHP water and dried at 70°C for 5 days.

Further pots were set up in the same way but left unplanted to measure gas productions in the bulk soil following flooding. Each pot was fitted with a rhizon solution sampler (Rhizosphere research products, Wageningen, Netherlands) with a 5-cm porous section and fitted with a Luer lock. The samplers were held vertically in the soil so that the porous section ran from 8.5 to 13.5 cm below the floodwater-soil boundary. At weekly intervals, solution was withdrawn and analysed for dissolved CO₂ (MI-720 electrode, Microelectrodes Inc, USA) and pH (MI-410 combination electrode, Microelectrodes Inc, USA). Redox potential was monitored with a Pt electrode. The composition of gas bubbles accumulated in the soil was monitored by periodically fitting over each pot a 3-dm⁻³ gas-tight bag fitted with a sampling port and agitating the pots to displace entrapped soil gases into the headspace. Samples of the headspace were withdrawn by syringe and analysed for CO₂ and CH₄ by gas chromatography (Cambridge Scientific Instruments 200 Series GC).

We estimate the pH buffer power (i.e., the amount of base required to produce unit increase in pH; bHS) of the submerged, reduced soil from the results of Affholder et al. (2017) who found with the same rice genotype and growth conditions as here that the pH averaged over the root zone increased by 0.34 pH units due to a net removal of H⁺ as H₂CO₂ through the roots of 11.0 mmol kg⁻¹ but offset by a net addition of 1.6 mmol H⁺ kg⁻¹ from the roots to balance excess intake of cations over anions. On the basis of the soil Fe (II) concentration, the addition of H⁺ in Fe (II) oxidation by the roots was far smaller. Hence, bHS = (11.0 - 1.6)/0.32 = 29 mmol·kg⁻¹·pH⁻¹.

### 2.3 X-ray CT imaging

Roots and gas bubbles in the pots were imaged using a Custom Nikon/XTEK Hutch X-ray CT scanner. The field of view was 8 cm in diameter and 5.6 cm in height, with the upper edge approximately at the base of the primary roots, 5 cm below the soil-floodwater boundary. The pots were scanned at 120 kV and 185 uA. A 1-mm copper filter was used to minimize beam hardening. A total of 3,001 angular projections through 360° were acquired at an exposure of 177 ms, with 32-frame averaging for each projection. The scan duration was 4.7 hr per sample, and the resulting voxel size was 40 μm (isotropic). Data were reconstructed using a filtered back-projection algorithm implemented in Nikon CTPro 3D, generating 32-bit volumes that were subsampled to produce a stack of two-dimensional eight-bit Tagged Image File Format files for each scan. A modest beam hardening correction was applied during reconstruction.

Gas bubbles were extracted from the data by 3D median filtering using an 8 × 8 × 8 voxel cubic kernel, then hysteresis thresholding, using the Fiji image analysis software (Schindelin et al., 2012). Aerenchymatous roots were extracted using a region-growth method (Keyes et al., 2013) followed by manual analysis of remaining roots in Avizo 9.0.0. The gas bubble geometry was subtracted from the root geometry to remove coclassified voxels. The spatial distributions of roots and gas were classified with respect to pot depth and radial distance from a vertical axis through the centre of the plants using code written in MATLAB 2018b (MathWorks, Massachusetts, USA).

We transformed the scanned root and gas data into volumetric spatial data (root length density, Lr, and volumetric gas content, Br) using the conversion that one voxel edge length was equivalent to 0.04 mm. Each scan was 5.8 cm (1,450 pixels) in depth, with approximately 5 cm of soil above the upper edge and 6 cm below the lower edge. The Lr and Br data were extrapolated over the entire depth by fitting three-dimensional Gaussian distributions to the pooled data for the three replicates for each planting density.
where $X$ is either $L_V$ or $\theta_G$ and $\varphi$, $\sigma$, and $\alpha$ are the corresponding fitting coefficients. Parameters were fitted in MATLAB using the fmincon function to minimize the square difference between the measurements and Equation (7).

### 2.4 Model parameterization

We solved Equation (1) for each of the three gases CO$_2$, CH$_4$, and N$_2$ subject to the stated boundary conditions and Equations (2)–(6) using standard numerical methods. We parameterized the model as follows.

First, we used preset values of the following parameters: (a) the three-dimensional distribution of $L_V$ obtained from the root images as described in the previous section; (b) $K_c$, $S_{CO_2,0}$, and $\alpha_{CH_4}$ in Equations (2)–(4) by running the model with no roots (i.e., no rhizodeposition and no gas venting through the roots) to fit the observed concentration of dissolved CO$_2$ and pH in the unplanted bulk soil and the ratio of CO$_2$ to CH$_4$ measured in entrapped gases displaced from the soil; and (c) all other variables, except $k_v$, $k_r$, and $k_v$, based on the experimental data and standard values for the constants and coefficients (Tables 1 and S1, Supporting Information).

We then fitted values of $k_v$, $k_r$, and $k_v$ by running the model to obtain the best agreement between our observed and predicted three-dimensional profiles of $\theta_G$ for each planting density, using the MATLAB fmincon function. A unique set of $k_v$, $k_r$, and $k_v$ values was found for the whole data set by minimizing the average of the fitting errors calculated for the individual replicate runs.

The rate of generation of CO$_2$ in the soil per unit soil surface was calculated from

$$J_S = S_{CO_2,0} \delta + k_v \frac{2}{R^2} \int_0^R L_V \cdot r \, dr \, dz.$$  

where $L$ and $R$ are the depth and radius of the soil volume, respectively. The flux through the roots was calculated from

$$J_R = k_r D_{CO_2} \frac{2}{R^2} \int_0^R \theta_G (C_{CO_2} - C_{CO_2,0}) \cdot r \, dr \, dz.$$  

The flux from the soil surface by ebullition was calculated from

$$J_E = k_v \frac{2}{R^2} \int_0^R \theta_G C_{CO_2} \cdot r \, dr \, dz.$$  

The flux from the soil surface by diffusion was calculated from

$$J_D = J_S - J_R - J_E.$$  

Copies of the experimental data and the source code for the model written in FORTRAN are available from https://doi.org/10.17862/cranfield.rd.7628870.
the straw during the harvest and to burn the straw produced after threshing (Fairhurst, Witt, Buish, & Dobermann, 2007; Greenland, 1997). The stubbles and roots are incorporated into the soil during land preparation for the following crop, and they decompose over the course of the crop. Inputs of carbon from roots—and hence $K_{\text{V}}$—are as soluble exudates, insoluble secretions, and detrital root material and are also highly variable. They depend on growth conditions, healthy plants tending to be less leaky (Rose et al., 2013; van der Gon et al., 2002), and on genotype, modern rice varieties bred for high grain yield having leaner and less leaky roots than traditional varieties (Jiang et al., 2017; Maurer, Kiese, Kreuzwieser, & Rennenberg, 2018; van der Gon et al., 2002).

The ratio of CH$_4$ to CO$_2$ production, $\alpha_{\text{CH}_4}$, depends on (a) the presence of inorganic oxidants and (b) the stochiometry of methanogenic soil organic matter decomposition and the resulting proportions of CH$_4$ produced from disproportionation of acetate versus reduction of CO$_2$ with H$_2$ (Reactions 2–4, Figure 1; Yao & Conrad, 2000). In general, the former dominates (Yao & Conrad, 2000), and $\alpha_{\text{CH}_4} = 1$ is typical (Kirk, 2004). A large proportion of the CH$_4$ flux will be oxidized to CO$_2$ by methanotrophic bacteria in the rhizosphere and oxic floodwater–soil interface; up to 95% of the root-mediated CH$_4$ flux is oxidized to CO$_2$ (Arah & Kirk, 2000; Cho, Schroth, & Zeyer, 2012; Hernández, Dumont, Yuan, & Conrad, 2015; Reid, Pal, & Jaffe, 2015; van Bodegom, Stams, Mollena, Boeje, & Leffelaar, 2001). The net root CO$_2$ flux will be correspondingly greater.

The root gas transmissivity, $k_T$, depends on such variables as aerenchyma volume fraction, the permeability of root tips and laterals, root architecture, and growth stage (Kirk, 2003; Yamauchi, Colmer, Pederson, & Nakazono, 2018). The value of $k_T$ will also influence the degree of aerobic CO$_2$ generation and CH$_4$ oxidation in the rhizosphere. Other things being equal, a high $k_T$ value reduces rather than enhances net CH$_4$ emission because it allows increased oxygenation of the rhizosphere (Arah & Kirk, 2000; Jiang et al., 2017). There is not much published information with which to judge our $k_T$ values directly. However, from the wealth of information on the root pathway for CH$_4$ emissions from rice, our root fluxes of CO$_2$ are highly plausible.

### 4.2 Mechanisms of CO$_2$ entry into the root

To reach the aerenchyma in the root cortex, dissolved CO$_2$ and HCO$_3^-$ in the soil solution must pass through the root wall and epidermal tissues. Under anoxic conditions in submerged soil, the rice root system develops a layer of suberized cells in the walls of primary roots starting 1–1.5 cm behind the root tip (Yamauchi et al., 2018). This layer is highly impermeable to O$_2$—and by implication to CO$_2$—and so restricts radial loss of O$_2$ to the soil and thereby allows a
greater length of root to be aerated (Yamauchi et al., 2018). The rice root system typically comprises coarse, aerenchymatous, primary roots with gas-impermeable walls conducting O₂ to short, fine, gas-permeable laterals, which have a much greater surface area per unit mass than the primary roots. Kirk (2003) shows that this architecture provides the greatest absorbing surface for nutrients per unit aerated root mass. The same argument would apply to the absorption of CO₂ by the root system. A further pathway for soil CO₂ into the aerenchyma may be via the basal stem tissue at the root–shoot junction below the soil surface (Pedersen, Pulido, Rich, & Colmer, 2011).

After crossing the root wall, the dissolved CO₂ in the root apoplast must pass through the epidermal tissue. The passive apoplastic route through the epidermis is obstructed by the Casparian strip and so CO₂ or HCO₃⁻ or both must cross the plasma membrane into the symplasm. Whereas uncharged CO₂ molecules can pass through cell walls passively, HCO₃⁻ anions cannot. This is problematic because there are no known membrane transporters for HCO₃⁻ in higher land plants (Bloemen, McGuire, Aubrey, Teskey, & Steppe, 2013; Poschenrieder et al., 2018; Shimono, Kondo, & Evans, 2019). A boron transporter, BOR1, is reported to be homologous to an animal HCO₃⁻ transporter (Takano et al., 2002), but there is as yet no evidence that it functions as such in plants. This implies that HCO₃⁻ must be converted into CO₂, which then diffuses to the cortex via the symplasm.

At the pH of the soil bulk in our experiment (7.0), 82% of the dissolved CO₂ (H₂CO₃* + HCO₃⁻) is in the form of HCO₃⁻. Removal of CO₂ from the soil close to root surfaces will tend to raise the soil pH (Section 4.5). But the root apoplast is generally acidified to some extent: Felle (2001) gives values below pH 6. At pH 6.5, the proportions of dissolved CO₂ and HCO₃⁻ are nearly equal, so the apoplastic–symplastic route will be greatly enhanced to the extent that the apoplast is acidified. We know of no studies of root apoplastic pH in rice. But given that, in general, the main form of N taken up in paddy soils is NH₄⁺, so that cation uptake exceeds anion uptake, the apoplast is likely to be acidified. Geilfus (2017) reviews methods for measuring apoplastic pH.

The uncatalysed CO₂ hydration–dehydration reactions, by which H₂CO₃ and hence HCO₃⁻ equilibrates with CO₂ (HCO₃⁻ + H⁺ = H₂CO₃ = CO₂ + H₂O), are slow, and so may be rate limiting for the apoplastic–symplastic pathway or degassing of CO₂ into the aerenchyma or both. The presence of carbonic anhydrase (CA), which catalyses the reactions, in the apoplast is therefore an important question. Cytosolic CA is ubiquitous in plant tissues (DiMario, Clayton, Mukherjee, Ludwig, & Moroney, 2017), but its presence in the apoplast is less certain (Savchenko, Wiese, Neimanis, Hedrich, & Heber, 2000).
4.3 | Fate of the CO2 in the root

Is the concentration of CO2 and associated HCO3− in the roots sufficient to be toxic? The soil CO2 concentration in our experiment was equivalent to P_{CO2} ≈ 20 kPa in the soil bulk but tenfold less than this at the root surface as a result of venting through the roots. Plant species well adapted to high P_{CO2} in the root zone, such as rice, can thrive at P_{CO2} values well above 20 kPa through mechanisms that are not well understood (Greenway et al., 2006). If the cytoplasm was in equilibrium with P_{CO2} = 20 kPa and the pH was maintained at the typical value of 7.5 through the biochemical and biophysical pH stats, then the cytoplasmic HCO3− concentration would be approximately 90 mM, which is above values at which metabolism is impaired (of the order of 50 mM or possibly as low as 10 mM for some enzyme systems—Greenway et al., 2006), whereas at P_{CO2} = 2 kPa, as calculated for the soil at the root surface, the HCO3− concentration would be only about 9 mM, which is in the normal range (2–20 mM) and well below toxic levels. This indicates that the rate of CO2 venting through the roots would be sufficient to avoid toxic concentrations in root cells.

In fact, the enhanced availability of CO2 in the roots may have a growth stimulating effect in rice by facilitating anaplerotic production of organic acids for amino acid synthesis (Balkos, Britto, & Kronzucker, 2010; Britto & Kronzucker, 2005). In general, the main form of N taken up by rice in submerged soils is NH4+, and virtually all the NH4+ is assimilated into amino acids in the roots before being transported to the shoots (Kronzucker, Siddiqi, Glass, & Kirk, 1999). This occurs via glutamine synthetase (GS), which catalyses the incorporation of NH4+ into the organic pool, and phosphoenolpyruvate carboxylase (PEPC), which fixes CO2 into oxaloacetate and malate so providing carbon skeletons for the GS pathway. In principle, if other factors are nonlimiting, increased CO2 supply in the roots would allow greater N assimilation.

The PEPC pathway might be a significant sink for root CO2. An upper estimate of the size of this sink can be got from the rate of N uptake by the roots with the crude assumption that all the N is taken up as NH4+ and assimilated via GS and PEPC. From the plant growth rate (0.45 g day^{−1} at 28 days after transplanting—Section 3.2) and N content (approximately 15 mg g^{−1}—Affholder et al., 2017), the rate of N uptake was approximately 0.48 mmol day^{−1}, which is less than 10% of the CO2 flux through the roots. In fact, a significant part of N uptake by rice in submerged soils is as NO3−, formed by nitrification of NH4+ in the rhizosphere (Kirk & Kronzucker, 2005), and most of the NO3− will be assimilated in the shoots rather than the roots (Kronzucker et al., 1999). We conclude the flux of CO2 through PEPC in the roots will be small compared with the net CO2 flux. This is consistent with the assumption implicit in the model that, at steady state, effectively all the CO2 entering the roots diffuses to the shoots via the aerenchyma (Equation 6).

4.4 | Fate of the CO2 reaching the shoot

Could recycling of root- and soil-derived CO2 through the roots to the shoots provide a source of CO2 for photosynthesis? The soil-derived CO2 flux through the plants was equivalent to approximately a third of the daily rate of photosynthesis, that is, 20% of the actual rate of photosynthesis given that the photoperiod was 13.5 hr. This suggests a large potential source for photosynthesis. We know of no data on this point for rice plants. However, measurements with emergent wetland plants such as Phragmites suggest sediment-derived CO2 accounts for less than 1% of the carbon fixed by the shoots (Brix, 1990; Constable & Longstreth, 1994; Singer, Esbel, Agami, & Beer, 1994). Although aerenchyma provides a continuous gas pathway between the roots and leaves, the stems of rice plants contain lenticels that allow gas exchange with the atmosphere in the lower part of the canopy (Yamauchi et al., 2018). So the bulk of the root-borne CO2 probably escapes from the aerenchyma before reaching the main photosynthetic tissue.
4.5 | Other implications

Removal of soil CO$_2$ through the roots has important implications for the chemistry of the rhizosphere. Removal of dissolved CO$_2$ and hence H$_2$CO$_3$ will tend to increase the rhizosphere pH. The maximum depletion of H$_2$CO$_3$ + HCO$_3^-$ by the roots (Figure 3) was 30 mM, that is, 21 mmol kg$^{-1}$ allowing for the soil water content and bulk density. Hence, from the pH buffer power of the soil ($b_{H^+} = 29$ mmol kg$^{-1}$, Section 2.2) the expected pH increase close to the roots is 0.7 units, that is, from 7.0 to 7.7. Such a pH change would substantially alter the solubility and hence plant availability of nutrients and toxicants (Kirk, 2004). For example, a pH increase in this range would make soil organic ligands more soluble and thereby solubilize soil Zn (Affholder et al., 2017). In “iron toxic” rice soils, where large concentrations of dissolved ferrous iron can severely damage the plants (Becker & Asch, 2005), H$^+$ consumption in CO$_2$ venting could moderate the acidification of the rhizosphere caused by ferrous iron oxidation (4Fe$^{2+}$ + O$_2$ + 10H$_2$O = 4Fe(OH)$_3$ + 8H$^+$) and so limit the impairment of cation uptake caused by acidification (Begg et al., 1994).

The likely importance of CA in facilitating CO$_2$ entry into the root and aerenchyma (Section 4.2) raises a possible link to the plant Zn nutrition. The active centre in all known plant CAs contains Zn (DiMario et al., 2017), and Zn-deficient plants can have impaired CA activity (Sasaki, Hirose, Watanabe, & Ohsughi, 1998). Consistent with this, Affholder et al. (2017) found less CO$_2$ venting through a rice genotype sensitive to soil Zn deficiency compared with a tolerant genotype.

What factors could be manipulated by plant breeding or crop management to influence soil CO$_2$ uptake by rice roots? The extent of aerenchyma development and gas barriers in the root wall will be important, both for CO$_2$ transmission and for oxidation of CH$_4$ to CO$_2$ in the rhizosphere; there are differences in both of these between rice genotypes (Yamauchi et al., 2018). There are also genotype differences in CA expression in rice (Xu, Zhang, Guan, Takano, & Liu, 2007).

5 | CONCLUSIONS

1. Venting through the roots of CO$_2$ formed in root and soil respiration is an important control on root and soil CO$_2$ concentrations in submerged wetland soils over a wide range of plant and soil conditions.

2. We measured rates of CO$_2$ uptake by roots equivalent to a third of the daily CO$_2$ fixation in photosynthesis. Without this venting through the roots, the concentrations of CO$_2$ and associated HCO$_3^-$ in root cells would have been well above levels known to be toxic to roots.

3. The removal of CO$_2$ and hence H$_2$CO$_3$ from the soil was sufficient to increase the rhizosphere pH close to the roots by 0.7 units. That is sufficient to solubilize or immobilize various nutrients and toxicants and potentially provides an explanation for genotype differences in tolerance of nutrient deficiencies and mineral toxicities.

4. The image-based mathematical modelling method that we used, linked to non-invasive X-ray CT imaging, is a powerful way of studying below-ground plant–soil interactions.

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ORCID

Guy J.D. Kirk https://orcid.org/0000-0002-7739-9772
Andrea Baghi https://orcid.org/0000-0002-9387-326X

REFERENCES


**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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**Table S1** Values of diffusion coefficients and Henry’s law constants at 25 oC (2). Also, apparent 1st dissociation constant of H2CO3, K1 = 4.45 x 10⁻⁷ mol dm⁻³; saturating water pressure, PH2O = 5 kPa; gas constant, R = 8.314 dm³ kPa K⁻¹ mol⁻¹

**Fig. S1** Measured and modelled results for the second replicate with 4 plants per pot.

**Fig. S2** Measured and modelled results for the third replicate with 4 plants per pot.

**Fig. S3** Measured and modelled results for the first replicate with 1 plant per pot.

**Fig. S4** Measured and modelled results for the second replicate with 1 plant per pot.

**Fig. S5** Measured and modelled results for the third replicate with 1 plant per pot.

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