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Overcoming constraints to measuring O2 diffusivity and consumption of intact roots

Juan de la Cruz Jiménez 1,* William Armstrong 2,3 Timothy D. Colmer 3,4 and Ole Pedersen 1,5

1 Department of Biology, University of Copenhagen, Copenhagen 2100, Denmark
2 Department of Biological Sciences, University of Hull, Hull HU6 7RX, UK
3 School of Agriculture and Environment, The University of Western Australia, Crawley, WA 6009, Australia
4 The UWA Institute of Agriculture, The University of Western Australia, Crawley, WA 6009, Australia
5 School of Biological Sciences, The University of Western Australia, Crawley, WA 6009, Australia

*Author for correspondence: juan.jimenezserna@bio.ku.dk

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Dear Editor,

Oxygen is essential for root growth, ion uptake, and cell maintenance and is obtained by radial diffusion from the soil, and/or longitudinally from the shoot system. In shoots, the O2 is sourced from photosynthesis or the aerial environment and moves into and along roots through the cortical gas spaces. Supply from the shoot dominates if roots are in an anaerobic environment and O2 will then diffuse outward from root to medium viz radial O2 loss (ROL) unless prevented by secondary O2-impermeable apoplastic barriers and O2 consumption in the epidermal-hypodermal layers. Oxygen availability to cells within the root is determined by their position, their O2 demands, and those of abutting tissues and by the resistances to diffusion along the supply path. It is sometimes possible to measure the respiratory demands of tissues in vitro using isolated segments of stele, or cortex, or combined peripheral cell layers (e.g. epidermis and hypodermal tissue; Armstrong et al. 1991; Aguilar et al. 2003). However, such data must be used with caution because of wound responses and the disruption to substrate supply and tissue connectivity. Measuring the diffusive resistance to O2 through tissues can be even more problematic. Longitudinal gas-phase diffusive resistance through cortical tissue may be readily calculable from gas-filled porosity values, but radial liquid-phase diffusive resistances into the stele or across the peripheral epidermal and hypodermal tissues are not easily measured (Garthwaite et al. 2008; Kotula and Steudle 2009). Here, we propose an innovative approach for measuring both the diffusive resistance and O2 consumption of individual peripheral tissue layers in intact roots. Clark-type O2 microsensors (Revsbech 1989) will be used to identify the O2 concentration deficits across individual cell layers in conjunction with a root sleev ing electrode (Armstrong and Wright 1975) that imposes a measurable O2 sink for determining the O2 flux passing through all the cell layers and not used in respiration (Fig. 1). Both measurements are essential components in the equations for deriving both the respiratory rate (M; mol O2 cm−3 s−1) and the apparent O2 diffusion coefficient (D; cm2 s−1) across a tissue cylinder (Supplementary File S1). Intact plants will be fitted in a 2-compartment chamber, where the gas composition of the shoot compartment can be regulated. This approach will allow detailed quantification of O2 dynamics within root tissues and the specific contribution of the different root cell layers to O2 diffusion impedance and respiratory O2 consumption.

Diffusion is the primary mechanism by which gases move within roots (Armstrong 1979). Oxygen can diffuse longitudinally (axially) or radially throughout and across the root (Fig. 2A). Longitudinal diffusion in roots is primarily through the cortical gas spaces (Fig. 2A) where O2 diffusivity is high (D_{O2}/air = 0.201 cm2 s−1 at 20 °C). Consequently, longitudinal
diffusive resistances, which are readily calculable based on the length ($L$), cross sectional area ($A_x$) and fractional porosity of the root ($\varepsilon$) (viz, $R_{\text{longitudinal}} = L/D_{O_2} \varepsilon A_x$; Armstrong and Armstrong 2014), are relatively low, particularly so in aerenchymatous roots (Fig. 2C). By contrast, much of the radial $O_2$ diffusion in roots is within cells and across abutting tissues in the liquid phase where diffusion coefficients are at least 10,000-fold smaller (e.g. $D_{O_2/H_2O} = 2.1 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ at 20 °C). Resistances per unit length of path in the liquid phase are therefore much greater than via the cortical gas space and made effectively a further 30-fold greater resistance because of low $O_2$ solubility in the liquid phase. In dense tissues (i.e. stele, epidermal, and exodermal layers), $O_2$ diffusion can be further reduced by wall deposits which are an additional physical barrier to $O_2$ diffusion, and by increased respiratory $O_2$ consumption per volume of tissue (Fig. 2C and D). Even without cell wall deposits or respiration, the radial resistance to $O_2$ diffusion is calculable as $R_{\text{radial}} = 30 \times r_c \log_e r_r/r_c / D_{O_2} A_c$ where $r_c$ is the radius of cortex, $r_r$ the root radius, and $A_c$ the surface area on $r_c$ within the segment (Armstrong and Armstrong 2014). For a hypothetical epidermal/hypodermal path of 0.0046 cm in a root segment of 1 cm length and 0.075 cm diameter (Figs. 2C and D) the resistance would be of ca. $2.98 \times 10^4 \text{ s cm}^{-3}$, whereas the comparable longitudinal diffusive resistance of a 10 cm, 30% aerenchymatous root of the same diameter would be $3.75 \times 10^4 \text{ s cm}^{-3}$. As a consequence, it is not surprising that $O_2$ can begin to rise in the stele of such an aerenchymatous root within 2 min of exposing the shoot to double the atmospheric $O_2$ concentration (Fig. 2B). Such contrasts in $O_2$ consumption and diffusivity strongly influence tissue $O_2$ supply across and along roots and are reflected in concentration gradients which can be accessed by $O_2$ microsensors (Fig. 2C and D).

Current developments in microsensing technologies (Pedersen et al. 2020) allow quantification of $O_2$ across root cell layers at sufficient resolution to identify changes in $pO_2$ at a nanomolar scale and at individual cell resolution (Fig. 2D). In addition, the development of genetically encoded $O_2$ biosensors responding to cellular changes in $O_2$ status, ATP levels, NAD redox dynamics or oxidative stress during plant acclimation to different concentrations of $O_2$ (Panicucci et al. 2020; Dalle Carbonare et al. 2023) could provide higher resolution of $pO_2$ to organelle level. However, the main limitation remains in the translation of such $O_2$ profiles/signals into diffusive resistances and respiratory $O_2$ consumption by individual cell layers. Any attempt to measure respiration should consider the effects of both $O_2$ resistance and consumption. For instance, bulk $O_2$ consumption rates of root segments with apoplastic cell wall barriers preventing radial $O_2$ diffusion increased 2- to 6-fold when these root sections were sliced open to allow $O_2$ diffusion to the entire root system, which is likely due to the removal of such barriers.

Figure 1. Diagram of the suggested experimental set-up. An intact individual adventitious root is positioned in a wire mesh and along with the remainder of the root system submerged in a deoxygenated agar solution. Shoots of plants are enclosed in a chamber with controlled concentrations of $O_2$ (i.e. 21 or 42 kPa). A polarographic platinum (Pt) root-sleeving $O_2$ electrode (cathode) is positioned at a specific location along the root and a Clark-type $O_2$ microsensor is further inserted through a hole in the Pt electrode and moved in microsteps from the deoxygenated medium down through the different root cell layers. The root-sleeving electrode will generate a fixed $O_2$ sink, and a constant readout of total ROL (i.e. outward $O_2$ diffusion) from the portion of the root within the electrode, while the microelectrode will measure $O_2$ concentrations in the distinct root cell layers. Created with BioRender.com.
root cells, in comparison with unopened root sections preventing O\textsubscript{2} diffusion into the root (Jiménez et al. 2021; Peralta Ogorek et al. 2023). Overlooking the important contribution of barriers to O\textsubscript{2} diffusion and individual respiratory O\textsubscript{2} consumption would lead to erroneous estimations of respiratory O\textsubscript{2} consumption rates, as a merely consequence of O\textsubscript{2} provision not reaching homogeneously the entire tissues.

Models for root aeration (Armstrong and Beckett 1987; Armstrong et al. 1991) represent the closest approximation to understanding how O\textsubscript{2} resistances and consumption in roots affect oxygen supply and distribution, but experimentally determined input values for tissue diffusivities and respiratory activities in roots are scarce. The method proposed here (see Supplementary File S1), will allow quantification of pO\textsubscript{2} across individual cell layers and the throughflow diffusion across these cell layers, which will allow both the respiratory rate and the apparent O\textsubscript{2} diffusion across each of the different cell layers of a root to be derived.

The acquisition of detailed information on radial differences in O\textsubscript{2} demand is essential for understanding root aeration, respiration, and nutrient uptake. High-resolution O\textsubscript{2} consumption of individual root cells would allow a

**Figure 2.** Oxygen dynamics within roots. Longitudinal and radial diffusion of O\textsubscript{2} (A), differences in pO\textsubscript{2} in the stele tissue when changing the pO\textsubscript{2} in the shoots (B), pO\textsubscript{2} profile across adventitious root of intact plants of *Urochloa humidicola* (C), and detailed pO\textsubscript{2} profile taken at the periphery of the root (D). Root characteristics including the number of cell files, stele area, gas-filled spaces, the chemical composition of cells, and the individual respiratory O\textsubscript{2} consumption determine radial and longitudinal O\textsubscript{2} diffusion and bulk tissue respiration. Asterisk keys in (A) indicate gas-filled spaces in roots. In (B), the shoot of entire plants of *U. humidicola* was sealed off in a chamber and the roots were placed in a glass chamber filled with stirred water at O\textsubscript{2} equilibrium (21 kPa). A Clark-type O\textsubscript{2} microelectrode was inserted in the stele tissue and the pO\textsubscript{2} in the shoot was changed from 21 to 42 kPa (dotted line) and then changed back to 21 kPa (short dash line). pO\textsubscript{2} profiles were taken in step sizes of 25 µm or 5 µm in (C) and (D), respectively, starting outside root surface (medium) and ending inside the root tissues. In (D), there is a clear efflux of O\textsubscript{2} from the cortex outward into the sclerenchyma but the steepness of the gradient is indicative of a very substantial diffusive resistance as does the steepness of the gradient from the medium into the exodermal/epidermal cells. If the medium around the root had been anaerobic, the combination of high diffusive resistance and respiratory demand would probably have presented an almost complete barrier to ROL to the medium. Vertical dotted lines in (D) indicate approximate boundaries of tissue layers. Ep = epidermis, Ex = exodermis, Sc = sclerenchyma.
comprehensive characterization of cell growth and energy-driven ion transport, where higher ATP requirements are probably needed in developing cells and at ion entry points located at exodermal and endodermal cells. Moreover, a complete understanding of O₂ dynamics in roots and the influence of root anatomy on respiratory O₂ consumption would allow the scientific community to characterize in detail plant responses to anoxic conditions (flooded soils or submergence), O₂ limitations during postharvest, or the role of O₂ in developmental processes (cf. Weits et al. 2019).

Author contributions
All authors designed this study. J.d.l.C.J. and O.P. conducted microsensor experiments. J.d.l.C.J. and W.A. drafted the letter and supplemental data equations. All authors revised and edited the letter.

Supplementary data
The following material is available in the online version of this article.
Supplementary File S1. Mathematical modeling to quantify resistances and respiratory O₂ consumption of individual root layers.

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Conflict of interest statement. None declared.

Data availability
All data supporting the findings of this study are available in the main paper and supplementary data.

References


