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Overcoming constraints to measuring O₂ diffusivity and consumption of intact roots

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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 sleeving 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. $D_{O_2/H_2O} = 2.1 \times 10^{-5}$ cm$^2$ 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 $R_{\text{radial}} = 30 \times r_c \log_e r_r/r_c/LD_{O_2}A_x$: where $r_c$ is the radius of cortex, $r_r$ the root radius, and $A_x$ the surface area on $r_r$ 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. $2.98 \times 10^4$ s cm$^{-3}$, whereas the comparable longitudinal diffusive resistance of a 10 cm, 30% aerenchymatous root of the same diameter would be $3.75 \times 10^4$ 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 cells, in comparison with unopened root sections preventing O₂ diffusion into the root (Jiménez et al. 2021; Peralta Ogorek et al. 2023). Overlooking the important contribution of barriers to O₂ diffusion and individual respiratory O₂ consumption would lead to erroneous estimations of respiratory O₂ consumption rates, as a merely consequence of O₂ 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 O₂ 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 pO₂ across individual cell layers and the throughput diffusion across these cell layers, which will allow both the respiratory rate and the apparent O₂ diffusion across each of the different cell layers of a root to be derived.

The acquisition of detailed information on radial differences in O₂ demand is essential for understanding root aeration, respiration, and nutrient uptake. High-resolution O₂ consumption of individual root cells would allow a
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


