

# A meta-analysis of plant tissue O<sub>2</sub> dynamics

Max Herzog<sup>A,\*</sup> , Elisa Pellegrini<sup>A,B</sup>  and Ole Pedersen<sup>A,C</sup> 

For full list of author affiliations and declarations see end of paper

**\*Correspondence to:**

Max Herzog  
The Freshwater Biological Laboratory,  
Department of Biology, University of  
Copenhagen, Universitetsparken 4,  
3rd Floor, Copenhagen 2100, Denmark  
Email: [herzogmax@bio.ku.dk](mailto:herzogmax@bio.ku.dk)

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## ABSTRACT

Adequate tissue O<sub>2</sub> supply is crucial for plant function. We aimed to identify the environmental conditions and plant characteristics that affect plant tissue O<sub>2</sub> status. We extracted data and performed meta-analysis on >1500 published tissue O<sub>2</sub> measurements from 112 species. Tissue O<sub>2</sub> status ranged from anoxic conditions in roots to >53 kPa in submerged, photosynthesising shoots. Using information-theoretic model selection, we identified ‘submergence’, ‘light’, ‘tissue type’ as well as ‘light × submergence’ interaction as significant drivers of tissue O<sub>2</sub> status. Median O<sub>2</sub> status were especially low (<50% of atmospheric equilibrium) in belowground rhizomes, potato (*Solanum tuberosum*) tubers and root nodules. Mean shoot and root O<sub>2</sub> were ~25% higher in light than in dark when shoots had atmospheric contact. However, light showed a significant interaction with submergence on plant O<sub>2</sub>, with a submergence-induced 44% increase in light, compared with a 42% decline in dark, relative to plants with atmospheric contact. During submergence, ambient water column O<sub>2</sub> and shoot tissue O<sub>2</sub> correlated stronger in darkness than in light conditions. Although use of miniaturised Clark-type O<sub>2</sub> electrodes has enhanced understanding of plant O<sub>2</sub> dynamics, application of non-invasive methods in plants is still lacking behind its widespread use in mammalian tissues.

**Keywords:** anoxia, flooding, hypoxia, internal O<sub>2</sub>, internal oxygen, light, O<sub>2</sub> dynamics, oxygen dynamics, submergence, tissue O<sub>2</sub>, tissue oxygen.

## Introduction

Following the discovery of O<sub>2</sub> (‘fire air’) in 1771, scientists have attempted to elucidate the interaction of O<sub>2</sub> with plants. In photosynthetic organisms, O<sub>2</sub> is produced as a by-product of photosynthesis, as electrons removed from H<sub>2</sub>O (generating O<sub>2</sub>) pass through PSII and PSI for the generation of NADP(H), subsequently reducing CO<sub>2</sub> into organic carbon (Blankenship 2002). Accumulation of O<sub>2</sub> inside plant tissues results in a concentration gradient driving a diffusive flux of O<sub>2</sub>. This flux may be to other parts of the plant (Armstrong 1979), the surrounding environment, or in some specialised wetland plants, even be driven by mass flow into other plant organs (Dacey 1980).

Plant O<sub>2</sub> dynamics are important for understanding processes crucial for plant functioning such as respiration, photosynthesis, and reactive O<sub>2</sub> species (ROS) production (Blokchina *et al.* 2003; Gibbs and Greenway 2003; Schmidt *et al.* 2018). As in most other organisms, O<sub>2</sub> in plants acts as electron acceptor in oxidative phosphorylation generating ATP (Millar *et al.* 2011). When O<sub>2</sub> is absent, plants can generate some ATP through glycolysis and anaerobic fermentation, although with much lower ATP yield per molecule of glucose (Bailey-Serres and Voeselek 2008). O<sub>2</sub> also leads to photorespiration, where Rubisco reacts with O<sub>2</sub> rather than with CO<sub>2</sub> to form RuPB in its oxygenase reaction. Photorespiration thus results in carbon loss, but also protects plants from photoinhibition (Kozaki and Takeba 1996). O<sub>2</sub> can generate ROS that are important molecules in cell signalling (Van Breusegem *et al.* 2008), but can also results in cell damage (Blokchina *et al.* 2003). More recently, the identification of an O<sub>2</sub> sensing mechanism in plants (Licausi *et al.* 2011), with an analogous O<sub>2</sub>-sensing pathway described for animal tissues (Holdsworth and Gibbs 2020), expanded the knowledge on plant adaptations to low O<sub>2</sub>. In addition, observations that O<sub>2</sub> acts as a signalling molecule in plant development

(Weits et al. 2019) has advocated a shift in perceiving low O<sub>2</sub> as a plant stressor to also view low O<sub>2</sub> as a constitutive, 'chronic' condition regulating plant growth and development (Weits et al. 2021).

The occurrence of low O<sub>2</sub> status in plant tissues is often overlooked, most likely due to photosynthetic O<sub>2</sub> production (Ast et al. 2012). Plants are sessile organisms, which requires them to adapt to environmental changes in their surroundings, including changes in O<sub>2</sub> availability (Ast et al. 2012). Flooding has profound effects on the O<sub>2</sub> status of plant tissue. Complete or even partial submergence affects plant O<sub>2</sub> status due to the 10 000-time slower gas diffusion in water than in air, lower solubility of O<sub>2</sub> in water than in air, light attenuation by flood waters and microorganisms depleting flooded soils of O<sub>2</sub> (Voeselek et al. 2006). Studies have also demonstrated that biotic and abiotic factors such as pathogen infection, temperature, salinity, and light conditions affect plant tissue O<sub>2</sub> status (Kumari et al. 2017; Chung et al. 2019; Koch et al. 2022a, 2022b). Thus, the lack of an active O<sub>2</sub> distribution system in plants, presence of non-photosynthetic plant tissues, and a number of biotic and abiotic factors affecting tissue O<sub>2</sub>, thereby resulting in substantial internal gradients in molecular O<sub>2</sub>.

Some plants possess mechanisms to control tissue O<sub>2</sub> status, such as aerenchyma providing a low resistance pathway for internal O<sub>2</sub> transport (Armstrong 1979). Certain plants also show adaptations restricting O<sub>2</sub> transport across tissues. For example, O<sub>2</sub> diffusion barriers in N<sub>2</sub> fixing root nodules limit the diffusive flux of O<sub>2</sub> to the root nodule, as O<sub>2</sub> would otherwise irreversibly inactivate nitrogenases responsible for N<sub>2</sub> reduction (Hunt et al. 1988). Another example of O<sub>2</sub> controlling mechanisms is the deposition of lignin and suberin in the cell wall along the outer part of a root restricting O<sub>2</sub> loss from roots into anoxic soils (Colmer 2003).

As plant organs consume and produce O<sub>2</sub> simultaneously, O<sub>2</sub> status within the same plant may vary substantially from organ to organ. For example, in *Sacocornia fruticosa*, root O<sub>2</sub> status was only 39% of the O<sub>2</sub> level in photosynthesising shoot tissues (Pellegrini et al. 2017). Moreover, O<sub>2</sub> status may vary between adjacent tissues, such as the stele of maize (*Zea mays*) roots being only 50% of the level in the root cortex over a distance of only 100 µm (Armstrong et al. 1994). Thus, plant tissue O<sub>2</sub> status can be expected to be dynamic in space and time, depending on plant organ, tissue type, and environmental conditions.

Over time researchers have used and continue to use contrasting approaches to determine molecular O<sub>2</sub> within plant tissues. Data used in this review include the earliest measurements of O<sub>2</sub> in plant tissues made using gas analysers, where a discrete gas sample is brought in contact with an absorbent (Thoday 1913; Laing 1940; Scholander 1947; Armstrong and Gaynard 1976; Erdmann and Wiedenroth 1988; Jordan and Whigham 1988), or injected into gas chromatographs (Spalding et al. 1979; Tjepkema and Cartica 1982). More recently, the development of polarographic

electrode sensors and O<sub>2</sub> optodes resulted in the development of microsensors allowing for O<sub>2</sub> measurements with better spatial and temporal resolution than gas sampling (Ast and Draaijer 2014). The development of these sensors has allowed for continuous O<sub>2</sub> measurements within tissues, in contrast to discrete measurements obtained by gas sampling.

As evident from the above, plant tissue O<sub>2</sub> status is likely to depend on plant organ, tissue type, developmental status, and environmental conditions. In the present study, we aimed at identifying factors controlling tissue O<sub>2</sub> status; e.g. important environmental conditions such as light and floods. For this, we extracted 1567 published measurements of vegetative plant tissue O<sub>2</sub> levels from the literature spanning 112 plant species, together with other parameters such as ambient O<sub>2</sub> levels, light conditions, and flooding status etc. We then conducted quantitative meta-analyses identifying factors controlling tissue O<sub>2</sub> status. We depict the overall patterns generated by this vast amount of data, but also highlight different plant strategies in maintaining O<sub>2</sub> homeostasis in subsequent, detailed analyses. While including the majority of plant tissues allowing for a general overview, we refer to other studies regarding details on O<sub>2</sub> status in seeds (Borisjuk and Rolletschek 2009) and fruits (Ho et al. 2014). We show that plant tissue O<sub>2</sub> status depend particularly on the types of plant tissue, light conditions, and plant submergence status. We also show that hypoxic conditions especially develop in dense tissues and some roots, while hyperoxic conditions are observed in submerged, photosynthesising tissues. Finally, we discuss the progress in methods to determine plant tissue O<sub>2</sub> status.

## Analytical approach

### Literature search

We performed a structured and systematic literature search to identify studies reporting O<sub>2</sub> measurements in vegetative plant tissues. The search was conducted using the Web of Science BIOSIS database applying Boolean operators, as explained in detail in the Supplementary materials. The search resulted in a final list of 129 studies published up to September 2022 used for this review. We extracted each observation of plant tissue O<sub>2</sub> status and entered it into a database alongside information of plant species, tissue type, light conditions, submergence status, treatment, O<sub>2</sub> measurement technique, tissue excisions status etc. if provided in the source. The list of studies used for data extraction is available in Supplementary materials. The database of plant tissue O<sub>2</sub> levels can be accessed from the Dryad data repository at <https://doi.org/10.5061/dryad.cnp5hqc8v>.

### Data handling

The studies used for this review provide a wide range of ways to measure and report levels of plant tissue O<sub>2</sub>. The following

steps describe how we performed data extraction for our meta-analysis.

1. O<sub>2</sub> levels reported as mm Hg, concentrations (mg L<sup>-1</sup>, μmol L<sup>-1</sup>), % of air equilibrium or % O<sub>2</sub> was converted into kPa O<sub>2</sub> as detailed in Supplementary materials.
2. When plants were subject to contrasting treatments (submergence, light/dark, pathogen infection etc.), reported values for each separate treatment were extracted.
3. When tissue O<sub>2</sub> was reported as tissue profiles (e.g. through a root cortex) the mean of these data points within each tissue was calculated and used for the analyses. When radial profiles were longitudinally distributed along an organ (stem/root), mean values from each radial profile were extracted separately.
4. When O<sub>2</sub> was reported in a time series (fx diurnally), the time series' maximum (usually daylight) and minimum (usually late night-time) values were extracted and used for analyses. On some occasions, fluctuating ambient night-time and daytime O<sub>2</sub> levels resulted in significant diurnal and/or nocturnal variations in tissue O<sub>2</sub> (Pedersen *et al.* 2016). In these cases, two to three values spread across the period were extracted (early, mid and end of night/day period) and used for the analyses. When O<sub>2</sub> was measured over long time series (days, weeks, months) reporting daily/weekly averages, all available data points were extracted (Eklund 2000). In the case of Sorz and Hietz (2008), we extracted all 103 data points but averaged the treatments and seasons.

In a few studies, tissue O<sub>2</sub> was sampled by extracting internal plant gasses in a liquid such as ambient flood water. Such studies where we suspected that air bubbles escaping from plants tissues had been in contact with surrounding medium (thus allowing for O<sub>2</sub> exchange) prior to measurements were discarded (e.g. Setter *et al.* (1987); Erdmann and Wiedenroth (1988); Stevens *et al.* (2002)). Studies where gas sampling was performed using syringes, preventing O<sub>2</sub> exchange with the surrounding medium, were included in the analyses.

For correlating submerged plant shoot tissue pO<sub>2</sub> with water column pO<sub>2</sub>, we identified 26 studies simultaneously reporting shoot tissue and water column pO<sub>2</sub> levels. Corresponding values of tissue and water column pO<sub>2</sub> were then extracted. For diurnal measurement series, two to three values spread across the period were extracted. When water column levels were manipulated, shoot values for each steady state level of water column pO<sub>2</sub> were extracted. To allow for effects of internal gas transport to be evident in the meta-analysis, only measurements from intact plants were used for this analysis.

## Statistical analysis

Data were analysed using GraphPad Prism 9 (GraphPad Software, La Jolla, CA, USA) or R (R Core Team 2022) for Windows statistical software. Normality of distributions was confirmed by visual inspections of residual plots and Shapiro–Wilk normality test, and homogeneity of variance by using F-test or Brown–Forsythe test ( $P > 0.05$ ). When data failed these assumptions, data was transformed, or a non-parametric test was chosen. Further details on statistical tests are given in the respective figure captions.

## Modelling

We evaluated the influence of environmental conditions, plant species and tissue type on plant O<sub>2</sub> status using an information-theoretic model selection approach. This explorative, data-driven approach is increasingly used when several predictors may be associated with a particular trait (Symonds and Moussalli 2011). The information-theoretic model selection approach differs from traditional null-hypothesis significance testing, where a hypothesis is first defined, and then accepted or rejected based on  $P$ -values (Symonds and Moussalli 2011). Each competing model predicts the outcome variable (in our case: plant tissue O<sub>2</sub> status) using predictors and their interactions (in our case, submergence, light, tissue type etc.). Thus, each competing model can be viewed as a different hypothesis of how predictors affect plant O<sub>2</sub>. Ranking the competing models allowed us to estimate which model best explains tissue O<sub>2</sub> status. We used a type of regression analysis, Linear Mixed-effects Model (LMM), to estimate the model parameters and their importance in controlling plant O<sub>2</sub> status. The use of mixed model allowed us to include plant species as a random factor, taking the variability in plant tissue O<sub>2</sub> related to species specific factors (e.g. aerenchyma formation) into account.

We compared all the possible predictors and combinations of interaction and selected the best model using the Akaike Information Criterion (AIC) (Burnham and Anderson 2004). AIC values derive meaning from comparing with AIC of other generated candidate models. By penalising the number of model predictors, AIC allows for the selection of the 'best' model based on fit (e.g. large  $R^2$ ) but also model complexity, and thus presents a compromise between fit and complexity. For details on applied R packages, data categorisation and diagnostic plot (Fig. S1), see Supplementary materials.

## Results and discussion

Our comprehensive analysis of 129 studies on 112 plant species, including 1567 reported tissue O<sub>2</sub> measurements, showed that tissue O<sub>2</sub> status ranged from anoxia<sup>1</sup> to more than

<sup>1</sup>Below detection limits which is approximately 0.007 kPa for O<sub>2</sub> microelectrodes (Revsbech 1989).

2.5-fold atmospheric equilibrium. Our data-driven model selection identified 'submergence', 'light', 'tissue type' and 'light × submergence' as key parameters affecting plant tissue O<sub>2</sub> status, and we further conducted detailed meta-analyses allowing us to quantify the effects of 'submergence', 'light', and 'tissue type' on tissue O<sub>2</sub> status.

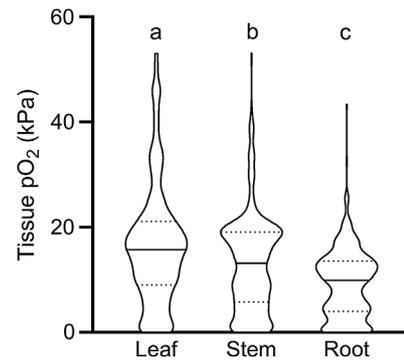
### 'Submergence', 'light', and 'tissue type' significantly affect plant tissue O<sub>2</sub> status

Our information-theoretic model selection identified 'submergence', 'light', 'tissue type' and 'light × submergence' interaction as the most important predictors of plant tissue status; i.e. these predictors had a relative variable importance of 1.0 in the only plausible linear mixed-effects model (Table 1). Meanwhile, the 'tissue type × light' interaction only had a relative variable importance of 0.8. This indicates that the 'tissue type × light' interaction was a weaker predictor of tissue O<sub>2</sub> than the other predictors.

Including species as a random factor increased the model fit. This is evident as marginal  $R^2$  was 0.40 without species as a random factor, compared with conditional  $R^2$  of 0.55 with the random factor included (Table 1). Following the data-driven identification of the predictors 'submergence', 'light', 'tissue type' and 'light × submergence' controlling plant tissue O<sub>2</sub> status, we continued by exploring our database of published O<sub>2</sub> status for patterns generated by these predictors.

### Plant O<sub>2</sub> status depends on plant tissue type

Our meta-analysis showed that O<sub>2</sub> status declined significantly from leaves to stems, and from stems to roots; i.e. median O<sub>2</sub> status was 15.7 kPa in leaves, 13.1 kPa in stems and 9.8 kPa in roots, respectively (Fig. 1). We interpret the significantly higher O<sub>2</sub> status in leaves to be caused in three ways: (1) by leaves producing endogenous O<sub>2</sub> via photosynthesis when in light; (2) by leaf stomata being able to vent



**Fig. 1.** O<sub>2</sub> partial pressures (pO<sub>2</sub>) in leaf, stem and root tissues. Numbers of replicate values ( $n$ ): 322, 633 and 404 for leaf, stem, and root tissues, respectively. Letters denote significant difference between medians (non-parametric Kruskal–Wallis test with Dunn's multiple comparisons,  $P < 0.05$ ). Truncated violin plot shapes illustrate observation frequency, the solid line the median and dashed line the quartiles. Data from experiments where the O<sub>2</sub> level of the atmosphere/submergence medium around the shoot was manipulated (i.e. atmosphere or submergence solution around shoot was purged with N<sub>2</sub>) was excluded in this figure.

leaf gas spaces, thus allowing for leaf O<sub>2</sub> status to remain closer to atmospheric levels than in more dense tissues without stomata; and (3) by leaves normally being in close contact with the atmosphere, in contrast to roots or rhizomes, which are in direct contact with O<sub>2</sub> consuming soils or sediments. Indeed, violin-plots showed a relatively high number of anoxic conditions in root tissues compared with leaves (Fig. 1). It should be noted that distribution of O<sub>2</sub> levels in roots may be influenced by a high number of wetland and aquatic plants in the published data underlying this analysis. In detail, 78% of the observations originated from aquatic and wetland plant species and 21% from terrestrial plants. Plant species inhabiting wetland and aquatic systems possess root traits such as aerenchyma that enhance internal aeration and thus ensure

**Table 1.** Result of the information-theory based model selection.

Model number	Model dependent variable	Intercept	Submergence	Light	Tissue type	Light × submergence	Tissue type × light	df	$R^2_c$	$R^2_m$	AIC	$\Delta$ AIC	Weight
Model 48	$\text{Sqrt}(pO_2)$	3.351	+	+	+	+	+	12	0.5485	0.4030	3198	0.00	0.694
	RVI		1.0	1.0	1.0	1.0	0.8	–	–	–	–	–	–
Model 16	$\text{Sqrt}(pO_2)$	3.330	+	+	+	+	–	10	0.5390	0.3983	3201	2.75	0.176
	RVI		1.0	1.0	1.0	1.0	–	–	–	–	–	–	–

The two best models are shown, but only model 48 was statistically plausible ( $\Delta$ AIC < 2). The models explained tissue O<sub>2</sub> status in relation to predictors 'light' (i.e. light or dark conditions), 'submergence' (i.e. shoot completely submerged, partially submerged, or shoot completely in air), 'tissue type' (root, stem or leaf) and interactions. Only studies clearly describing light, submergence and tissue types were included (101 studies). Data from experiments where tissues were excised or the O<sub>2</sub> level of the atmosphere/submergence medium around the shoot was manipulated (i.e. atmosphere or submergence solution around shoot was purged with N<sub>2</sub>) was excluded. This filtering resulted in the inclusion of data from 92 species, and 'plant species' was included as random factor. Tissue O<sub>2</sub> partial pressures (pO<sub>2</sub>) were square root transformed prior to the analysis. Intercept, df, marginal  $R^2_m$  (variance explained by fixed factors) and conditional  $R^2_c$  (variance explained by both fixed and random factors), Akaike information criterion (AIC) and  $\Delta$ AIC values, model weight (Weight) and relative variable importance (RVI) are reported. '+' indicates if a predictor was included in the respective model.

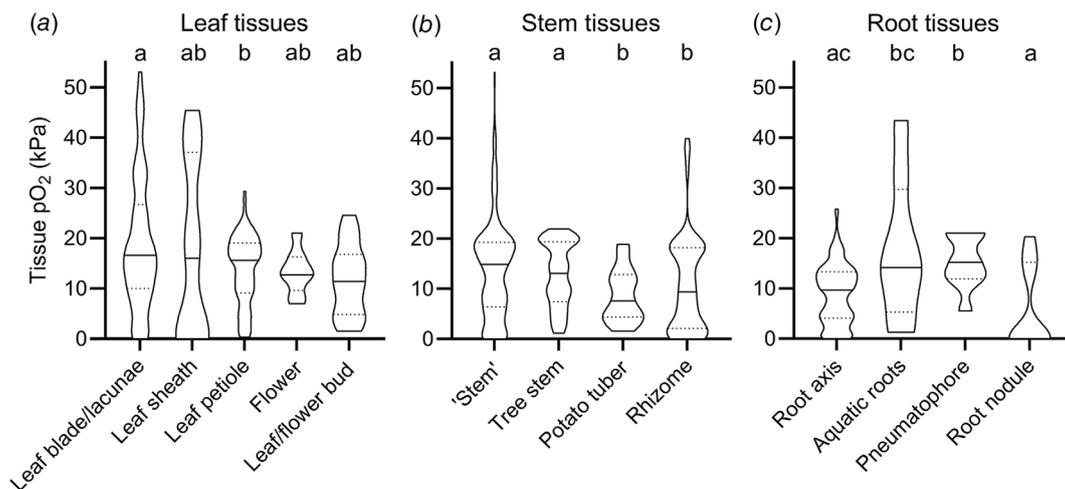
adequate tissue  $O_2$  supply for root functioning, whereas terrestrial plants typically lack such traits leading to  $O_2$  depletion in flooded soils (Armstrong 1979). The distribution of  $O_2$  levels reported in Fig. 1 should therefore be treated with caution when inferring a 'true' distribution of  $O_2$  in all plants.

The broad categorisation of tissue type into leaf, stem and root in Fig. 1 does not do justice to the vast range in morphology and function of different tissue types within these main categories. For example, the category of roots encompasses diverse tissues such as root tips in anoxic soils, mangrove pneumatophores (acting as pathways for atmospheric  $O_2$  inlet), aquatic adventitious roots and other contrasting root tissues. For example, two very high root tissue  $O_2$  levels (43.2 and 32.5 kPa, Fig. 1) were from chlorophyll containing, photosynthesising aquatic adventitious roots of *Meionectes brownii* (Rich *et al.* 2013). In addition to the broad categories in Fig. 1, we therefore also categorised measurements from leaf, shoot and root tissues into several subcategories (Fig. 2).

In the leaf tissues subcategories  $O_2$  levels were relatively similar compared with the vast variation in stem and root tissues; i.e. median  $O_2$  status only ranged from 11.4 to 16.8 kPa (Fig. 2a). The only significant difference was found between median leaf blades (16.8 kPa) and leaf petioles (15.2 kPa). There was no significant difference between the remaining leaf categories (leaf sheath, flower, and bud

tissues), most likely due to a low number of  $O_2$  observations in these tissues ( $n < 14$ ) compared with leaf blades and petioles ( $n > 102$ ).

Tissue  $O_2$  status of stems showed larger variation than leaf tissue and was significantly influenced by stem tissue type. Hence,  $O_2$  status in stem tissues was lower in thick, belowground, and predominantly respiring tissue types, compared with porous and photosynthesising tissue types (Fig. 2b). In stems, median  $O_2$  status in tissues such as culms, peduncles, stolons and stems of herbaceous plants (14.9 kPa, designated as 'stems' in Fig. 2b) and tree stems (13.1 kPa) were significantly higher, than in rhizomes (9.4 kPa) and the dense storage organ of *Solanum tuberosum* (7.6 kPa, designated as potato tuber in Fig. 2b). Thick and dense tissues showing high resistance across the diffusion path are prone to internal  $O_2$  gradients, especially if respiratory demand for  $O_2$  is large (Armstrong 1979; Colmer and Greenway 2005). Indeed, 32 g potato tubers experienced relatively low central  $O_2$  status of 1.5 kPa compared with 7 kPa in 8 g small tubers (Geigenberger *et al.* 2000). Another example of low internal  $O_2$  status in dense stem tissues results from radial profiling across dense succulent stem tissues of submerged *Halosarcia pergranulata* using an  $O_2$  microsensor (Pedersen *et al.* 2006). Here, internal  $O_2$  declined to 1.5 kPa compared with 21 kPa in the submergence solution. Respiration rates also affect plant tissue  $O_2$ , exemplified in potatoes stored at 22–25°C showing



**Fig. 2.**  $O_2$  partial pressures ( $pO_2$ ) in (a) leaf, (b) stem and (c) root tissue. In (b) 'Stem tissues' encompass stems, culms, stem internodes, peduncles, or stolon tissues but not rhizomes, storage organs (tubers and corms) and woody tree stems. Tree stems are woody stems of beech (*Fagus sylvatica*), Norway spruce (*Picea abies*), olive (*Olea europaea*), red maple (*Acer rubrum*), eastern hemlock (*Tsuga canadensis*), Northern red oak (*Quercus rubra*), black poplar (*Populus nigra*) and American ash (*Fraxinus americana*). Numbers of replicate values ( $n$ ): leaf blade/lacunae (102), leaf sheath (13), leaf petiole (125), flower (11), leaf/flower bud (14), 'Stem' (306), tree stem (97), potato tubers (28), rhizome (135), root axis (320), aquatic roots (8), pneumatophore (9), root nodule (15). Letters denote significant difference between medians (non-parametric Kruskal–Wallis test with Dunn's multiple comparisons,  $P < 0.05$ ). Truncated violin plot shapes illustrate observation frequency, the solid line the median and dashed line the quartiles. Data from experiments where the  $O_2$  level of the atmosphere/submergence medium around the shoot was manipulated (i.e. atmosphere or submergence solution around shoot was purged with  $N_2$ ) was excluded in this figure.

less than half of the tissue  $O_2$  compared with potatoes stored at 5–11°C (Stiles 1960). In summary, stem tissue  $O_2$  was particularly low in thick, dense, or predominantly respiring tissues such as succulent tissues or belowground rhizomes.

In stark contrast to potato tubers and succulent stems, median  $O_2$  was significantly higher in the other stem categories. Plant stems may contain lysigenous or schizogenous aerenchyma, facilitating gas exchange via interconnected air spaces that enhance  $O_2$  diffusion due to the 10 000-fold higher diffusion coefficients in gas than in liquid phase (Armstrong 1979). Since diffusion is a very slow process over long distances, some specialised wetland plants can induce ‘internal winds’ (pressure driven, convective gas flow) within connected air spaces allowing for effective  $O_2$  transport over long distances (Dacey 1980). For example,  $O_2$  in submerged culms of the sedge *Eleocharis sphacelata* remained close to atmospheric levels of 20 kPa down to 2.7 m of water depth during daytime where convective flow was operating, compared with <10 kPa during the night where convective flow is absent (Sorrell and Tanner 2000). This was a result of pressurised flow of gas from influx culms to efflux culms of up to 5.8 mL min<sup>-1</sup>, supplying immersed tissues with atmospheric  $O_2$ .

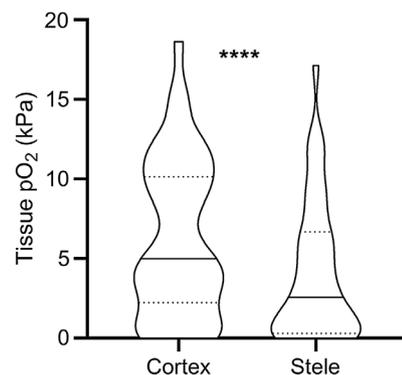
Root tissue  $O_2$  showed even higher range in median  $O_2$  status than leaf and stem tissues. Median  $O_2$  reached 15.2 kPa in pneumatophores but was 0 kPa in root nodules (Fig. 2c). In root nodules, symbiotic bacteria convert  $N_2$  gas into ammonia to be used in amino acid biosynthesis. Since the process of nitrogen fixation is highly  $O_2$  sensitive, root nodules have evolved mechanisms such as leghaemoglobin to regulate free  $O_2$  inside the nodule (Downie 2005). Indeed, the few observations of significant  $O_2$  inside root nodules belong to experiments where nodules were inoculated with mutant bacteria strains, some of which were unable to infect plant cells (Masepohl et al. 1993; Romanov et al. 1995). In contrast, the median  $O_2$  level in pneumatophores was significantly higher (15.2 kPa) than in the root axis or root nodules. Pneumatophores are lateral roots known from plants growing in anoxic mudflats such as mangroves (e.g. *Avicennia marina*), where they exhibit negative geotropism thereby growing upward. When reaching the sediment surface,  $O_2$  can pass through small bark openings (lenticles) and diffuse into the belowground root system. Other root tissues showing high  $O_2$  status were photosynthesising aquatic roots (Rich et al. 2011). Here,  $O_2$  status were as high as 43.2 kPa (median of 14.2 kPa, Fig. 2c).  $O_2$  may build up to such high levels in submerged, photosynthesising tissues due to physical restrictions in gas diffusion preventing photosynthetic  $O_2$  to diffuse out of the tissues and into the surrounding water.

### $O_2$ gradients within plant tissues

Obtaining  $O_2$  status with high spatial resolution has also revealed that  $O_2$  status vary significantly not only between but also within tissue types. Such data has mainly been obtained using Clark-type  $O_2$  microelectrodes. With a tip diameter of

down to 3  $\mu\text{m}$  (Weits et al. 2019) the electrode can be positioned in delicate tissues such as roots, even in field conditions. Our meta-analysis of literature data from studies reporting 40 paired root measurements of stele and cortex  $O_2$  status, reveal that mean  $O_2$  status in the root stele was 66% of the cortex  $O_2$  status (Fig. 3). The root stele is known to be more metabolically active compared with cortex tissues; e.g. for banana (*Musa* spp.) roots, a 6-fold difference in respiration rate was found (Aguilar et al. 2003). In addition to displaying high respiration rates, stele tissues only contain relatively few air spaces that further increases the  $O_2$  demand per volume of root tissue. In contrast, the root cortex may contain large air spaces caused by aerenchyma formation, allowing for longitudinal flow of  $O_2$  from the shoot or atmosphere toward the root tip (Armstrong 1979). Thus, the resistance to  $O_2$  diffusion increases in the dense stele compared with the cortex. The high respiration rates and low diffusivity in dense tissues result in the lower  $O_2$  status within root steles, as supported by modelling results (Armstrong et al. 2000).

In addition to root tissues, shoot tissues may also exhibit internal  $O_2$  gradients. Molecular  $O_2$  inside the shoot apical meristem of *Arabidopsis thaliana* (3.6–8.4 kPa) compared with the surrounding stem tissue (13–19 kPa) regulated leaf production, with hypoxia inhibiting proteolysis of an N-degron-pathway (Weits et al. 2019). The observed gradient in the shoot apex of 15 kPa developed over a distance less than 50  $\mu\text{m}$ . Meanwhile,  $O_2$  within the 2-mm thick leaf petioles of *Rumex palustris* was much more homogeneous, probably owing to the large number of internal gas spaces (aerenchyma content of >20%) and underwater photosynthesis of the green petioles (Mommer et al. 2004). Corresponding to a longitudinal decline of  $O_2$  in roots (discussed below), profiles along the leaf petioles of *R. palustris* exhibited  $\sim 3$  kPa



**Fig. 3.**  $O_2$  partial pressures ( $pO_2$ ) in root cortex and stele tissues reported in the literature. Values were extracted from 13 published studies where  $O_2$  microsensors were driven through both tissues (i.e. matched pairs). \* denotes statistical significant difference between means (paired t-test on square-root transformed data,  $P < 0.0001$ ,  $n = 40$ ). Truncated violin plot shapes illustrate observation frequency, the solid line the median and dashed line the quartiles. Mean  $pO_2$  was 6.2 kPa in the cortex and 4.1 kPa in the stele, respectively.

longitudinal O<sub>2</sub> decline from a position close to the O<sub>2</sub> source (leaf) to O<sub>2</sub> sink (roots) (Mommer *et al.* 2004).

Plant tissues also show significant longitudinal variation in internal O<sub>2</sub> status. In roots, the degree of longitudinal O<sub>2</sub> diffusion from the shoot (O<sub>2</sub> source) via the root base to the root tip (O<sub>2</sub> sink) is crucial for plant functioning when roots grow in O<sub>2</sub> free media such as soils with excess water (Armstrong 1979). Plant adaptations and acclimation enabling a continuous supply of shoot-derived molecular O<sub>2</sub> to the metabolically active root tip have therefore been thoroughly studied. Key traits such as aerenchyma formation (constitutive as well as inducible) and induction of barriers to radial O<sub>2</sub> loss, which prevent O<sub>2</sub> leaking from the root axis into anoxic soils, allow for continued root aeration despite the anoxic soil environment (Colmer 2003). To evaluate the effect on root aeration of a barrier to radial O<sub>2</sub> loss and aerenchyma formation, one can compare O<sub>2</sub> status of roots grown in a stagnant, deoxygenated nutrient solution mimicking a flooded soil (Wiengweera *et al.* 1997) with roots grown in aerated nutrient solution (mimicking a drained soil). Most wetland species will further induce aerenchyma and a barrier to radial O<sub>2</sub> loss only in the former condition. For example, in *Zea nicaraguensis*, root apical O<sub>2</sub> status was 8.7 kPa in roots formed in stagnant, deoxygenated nutrient solutions compared with 0.47 kPa in roots from aerated nutrient solution when measured in roots in a severely hypoxic solution (Pedersen *et al.* 2021). In the roots formed in stagnant, deoxygenated nutrient solution, a barrier to radial O<sub>2</sub> loss was induced, and root aerenchyma was 1.4–fold of that under aerated conditions (Pedersen *et al.* 2021). Thus, in this concrete example, aerenchyma and the barrier to radial O<sub>2</sub> loss resulted in better O<sub>2</sub> status of the roots formed in the stagnant, deoxygenated nutrient solution.

In order to isolate the effect of the barrier to radial O<sub>2</sub> loss on root aeration without shoot-derived O<sub>2</sub> and varying root lengths impeding interpretations, a recent study reported radial O<sub>2</sub> intrusion into submerged root segments of rice (*Oryza sativa*) (Peralta Ogorek *et al.* 2021). Here, maximum O<sub>2</sub> status within the root segments with a weak barrier to radial O<sub>2</sub> loss differed 54-fold from roots with a tight barrier, when incubated with 21 kPa O<sub>2</sub> in the surrounding water (Peralta Ogorek *et al.* 2021). The strong effect of the barrier to radial O<sub>2</sub> loss on root O<sub>2</sub> status underlines the importance of species-specific adaptations and/or acclimations for O<sub>2</sub> status in plant tissues.

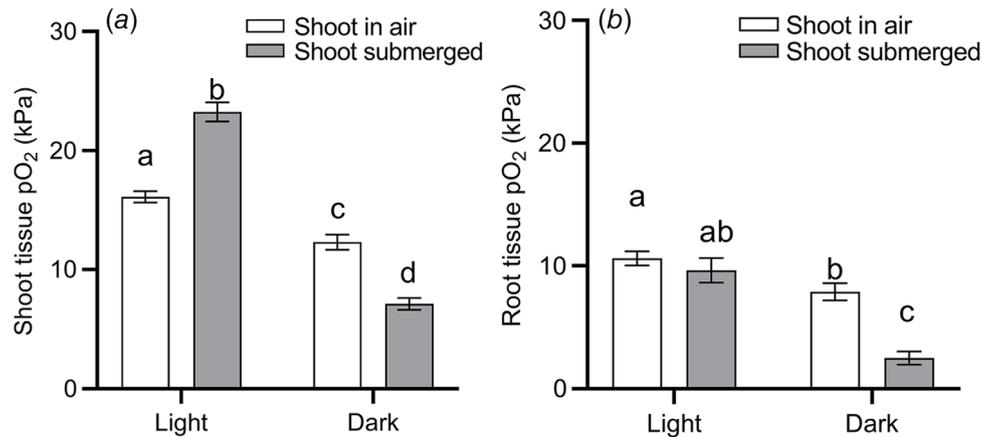
In conclusion, both root and shoot tissues may show substantial radial and longitudinal internal O<sub>2</sub> gradients. The gradients depend on tissue density, respiration activity, plant adaptations and/or acclimations such as aerenchyma and radial O<sub>2</sub> loss barrier formation, and environmental conditions.

### O<sub>2</sub> status in submerged plant tissues – the importance of light

Submergence is a compound stress to plants, brought on by several effects. For one, the 10 000–fold slower gas diffusion

in liquid compared with gas impedes the diffusive supply from the environment of O<sub>2</sub> for respiration and CO<sub>2</sub> for photosynthesis (Jackson and Drew 1984). As floodwaters absorb and scatter incoming light, photosynthesis may be hampered even further (Colmer *et al.* 2011). Build up of the volatile phytohormone ethylene may induce leaf senescence as chlorophyll is degraded (Voeselek and Bailey-Serres 2015). Thus, the capacity for underwater photosynthesis and therefore O<sub>2</sub> production generally declines with time of submergence for terrestrial vegetation (Colmer *et al.* 2011). Indeed, our data-driven model-selection approach identified both ‘submergence’ and ‘submergence × light’ interaction as factors that significantly affect plant tissue O<sub>2</sub> status (Table 1). It should be noted that the identification of ‘submergence’ over other environmental conditions could be due to a relatively large number of studies focusing on plant tissue O<sub>2</sub> in submerged plants (52% of observations used for model selection), compared with plants subject to drought, nutrient limitations, or pathogen infections. Nevertheless, the effect of submergence on plant tissue O<sub>2</sub> *per se* was validated by the model selection. To further quantify the effect of light and submergence on plant tissue O<sub>2</sub> status, we filtered data from the literature where O<sub>2</sub> was measured inside intact plants during complete submergence or with atmospheric contact (drained with shoot in air, waterlogged with shoot in air, or partial submerged shoot), and in light or dark conditions. We excluded data from experiments where the O<sub>2</sub> level of the atmosphere/submergence medium around the shoot was manipulated (i.e. atmosphere or submergence solution around the shoot was purged with N<sub>2</sub>), as this would otherwise mask any light or submergence effects. This analysis underlined the vast importance of light particularly for shoot tissues (Fig. 4). Shoots in air contained 24% less O<sub>2</sub> in dark than in light, most likely owing to the lack of photosynthetic O<sub>2</sub> under dark conditions. The effect of light on tissue O<sub>2</sub> was also evident in root tissues, where mean O<sub>2</sub> status declined from 10.6 kPa in roots of plants in light to 7.9 kPa in roots of plants under dark conditions.

The effect of complete submergence on shoot tissue O<sub>2</sub> status was strongly light dependent. In the presence of light, submergence resulted in a significant increase (44%) in shoot tissue O<sub>2</sub> status, compared with shoots with air contact (Fig. 4a). In contrast, when the entire shoot was submerged in dark conditions, submergence resulted in a significant decline (42%) in O<sub>2</sub> status. Hence two-way ANOVA showed a significant ‘light’ and ‘submergence × light’ interaction. We interpret the submergence-induced increase of shoot tissue in light as a build up of O<sub>2</sub> deriving endogenously from underwater photosynthesis, or to a lesser extent, from floodwaters containing high levels of O<sub>2</sub>. O<sub>2</sub> produced in shoot tissues by underwater photosynthesis is likely to build up as stomatal closure, physical boundary layers and slow outward diffusion of O<sub>2</sub> into the aquatic environment hampers gas exchange between the shoot and its surroundings (Sand-Jensen *et al.* 2005; Pedersen *et al.* 2006, 2016).



**Fig. 4.** O<sub>2</sub> partial pressures (pO<sub>2</sub>) in (a) shoot tissues and (b) root tissues during complete submergence ('shoot submerged', filled bars) or the shoot in air ('shoot in air', i.e. drained conditions, waterlogging and partial submergence, open bars) in light or dark conditions. Data were extracted from 117 studies where O<sub>2</sub> was measured inside intact plants (i.e. excluding studies where O<sub>2</sub> was measured within excised tissues) and where the shoot was clearly stated as being in light or dark conditions. Data was excluded when the O<sub>2</sub> level of the atmosphere/submergence medium around the shoot was manipulated (i.e. atmosphere or submergence solution around the shoot was purged with N<sub>2</sub>). (a) Letters denote statistically significant differences between means ( $P < 0.05$ , Tukey *post hoc* test;  $n = 201, 226, 106$  and  $221$  for columns from left to right) following 2-way ANOVA showing a significant light ( $P < 0.0001$ ) and submergence  $\times$  light interaction ( $P < 0.0001$ ). (b) Letters denote statistically significant differences between means ( $P < 0.05$ , Tukey *post hoc* test;  $n = 115, 48, 56$  and  $31$  for columns from left to right) following 2-way ANOVA showing a significant light ( $P < 0.0001$ ), submergence ( $P = 0.0001$ ) and submergence  $\times$  light interaction ( $P = 0.0078$ ). Bars show mean and error bars s.e.m.

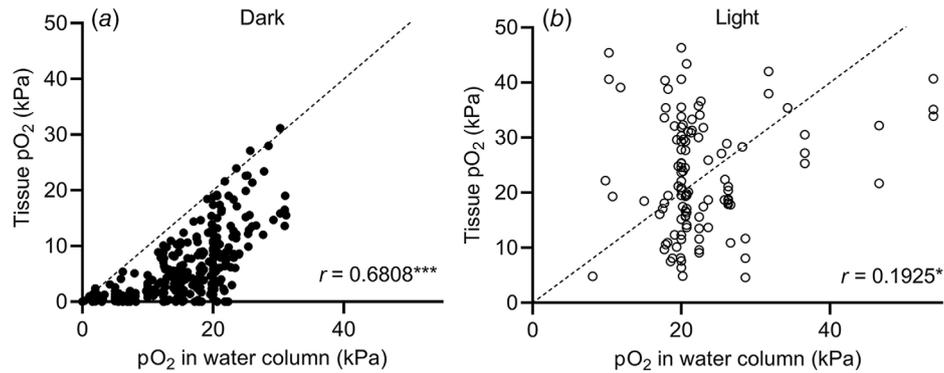
In contrast, in shoots with atmospheric contact, O<sub>2</sub> deriving from photosynthesis would be vented into the surrounding air. The importance of light on plant tissue O<sub>2</sub> *in situ* is nicely exemplified by diurnal leaf O<sub>2</sub> and irradiance measurements in seagrass (*Thalassia testudinum*) (Koch et al. 2022a). Here, a decline in daytime irradiance of  $\sim 1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  from passage of clouds resulted in a rapid decrease in leaf O<sub>2</sub> status from 45 to 27 kPa.

The submergence-induced increase in shoot tissue O<sub>2</sub> observed in Fig. 4a could also be due to high levels of O<sub>2</sub> in floodwaters. High levels (>30 kPa) of O<sub>2</sub> in natural floodwaters have been observed in marine (Pedersen et al. 2016) and freshwater (Rich et al. 2013) ecosystems. To test how floodwater O<sub>2</sub> levels affect plant tissue O<sub>2</sub>, we performed a correlation analysis of data from studies simultaneously measuring plant and floodwater O<sub>2</sub> levels (Fig. 5). Correlation between shoot tissue O<sub>2</sub> levels and water column O<sub>2</sub> levels was significant in both cases but stronger in the dark ( $r = 0.6808$ , Spearman,  $P < 0.0001$ ) than in light ( $r = 0.1925$ , Pearson,  $P = 0.0384$ ). The weaker correlation in light than in the dark indicates that production of O<sub>2</sub> in underwater photosynthesis provides the plant with an alternative O<sub>2</sub> source. As net underwater photosynthesis produces excess endogenous O<sub>2</sub>, the plant is less likely to experience the same O<sub>2</sub> level as the surrounding water column. In contrast, plants submerged in the dark rely on their immediate surroundings as O<sub>2</sub> source, and hence the correlation is stronger under dark conditions

than in the light. Since plant respiration consumes O<sub>2</sub>, points cluster below the line of equality in the dark (Fig. 5), but not in light where photosynthesis can elevate tissue O<sub>2</sub> to above water column O<sub>2</sub>.

Interestingly, three measurements from the leaf lacunae of the small isoetid *Lobelia dortmanna* (Møller and Sand-Jensen 2011) showed anoxic conditions in the shoot but high levels of floodwater O<sub>2</sub> (21–22 kPa) (Fig. 5). The reason why floodwater O<sub>2</sub> did not diffuse into the leaves of *L. dortmanna* most likely relates to its strategy for CO<sub>2</sub> acquisition. *L. dortmanna* is found in oligotrophic soft water lakes, where underwater photosynthesis is often carbon limited. Thick leaf cuticles therefore reduce permeability to CO<sub>2</sub> (and O<sub>2</sub>), helping *L. dortmanna* to acquire sediment-derived CO<sub>2</sub> for underwater photosynthesis via its root system. In the experiments by Møller and Sand-Jensen (2011), the sediment was enriched with organic matter, resulting in sediment O<sub>2</sub> depletion. Due to the lack of a barrier to radial O<sub>2</sub> loss in the root, and continuous internal air lacunae, O<sub>2</sub> was readily lost from the plant to the sediment. Meanwhile, the thick leaf cuticle restricted O<sub>2</sub> exchange with oxygenated floodwater. These observations nicely underline how plant tissue O<sub>2</sub> dynamics follow general trends but may deviate if habitats require special adaptations.

Continuous O<sub>2</sub> measurements using microelectrodes have revealed severe hyperoxia of >40 kPa O<sub>2</sub> (Sand-Jensen et al. 2005; Pedersen et al. 2006, 2016; Rich et al. 2013; Koch et al. 2022a) in submerged wetland and aquatic plants. The highest



**Fig. 5.** O<sub>2</sub> partial pressures (pO<sub>2</sub>) in submerged tissues vs O<sub>2</sub> levels in the surrounding water column under (a) dark or (b) light conditions with the line of equality (identity line). Values were extracted from 26 studies. O<sub>2</sub> values are from freshwater plants (*Lobelia dortmanna*, *Littorella uniflora* and *Isoetes australis*), seagrasses (*Cymodocea nodosa*, *Thalassia testudinum*, *Zostera marina*, *Zostera muelleri*, *Syringodium foliforme*, *Posidonia sinuosa*, *Thalassia hemprichii* and *Enhalus acoroides*) and wetland plants (*Suaeda maritima*, *Glyceria fluitans*, *Rumex palustris*, *Halosarcia pergranulata*, *Meinocetes brownii*, *Spartina anglica* and *Oryza sativa*) where O<sub>2</sub> in submerged tissues was measured simultaneously with water column O<sub>2</sub>. In experiments where diurnal variations were recorded, diurnal minimum and maximum values were extracted. For Pedersen *et al.* (2016), tidal waters caused the water column O<sub>2</sub> to fluctuate so O<sub>2</sub> measurements at the start, middle and end of the dark and light period, respectively, were extracted. Tissue types were leaves, leaf sheaths, rhizomes, stems, leaf meristems and leaf petioles but not roots. (a) Tissue and water column pO<sub>2</sub> recorded under dark conditions (at night in field experiments, or without day light/artificial light in laboratory experiments). Spearman correlation coefficient = 0.6808,  $P < 0.0001$  ( $n = 325$ ). (b) Tissue and water column pO<sub>2</sub> recorded under light conditions (during daytime in field experiments, or with artificial light in laboratory experiments). Pearson correlation coefficient = 0.1925,  $P = 0.0384$  ( $n = 116$ ).

non-manipulated plant tissue O<sub>2</sub> level reported in the literature (53.1 kPa) was measured in *Thalassia hemprichii* subjected to submergence by tidal waters and high radiation (2000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) (Pedersen *et al.* 2016). Hyperoxia in submerged vegetation coincides with high irradiation and may even intermit with night time hypoxia (Sand-Jensen *et al.* 2005; Pedersen *et al.* 2006, 2016). High levels of O<sub>2</sub> may result in the formation of ROS and increased photorespiration; however, the degree of tissue damage and plant mechanisms to tolerate hyperoxia remain much less studied than for hypoxia.

In our meta-analysis of tissue O<sub>2</sub> status, we have attempted to summarise the current state of knowledge on plant O<sub>2</sub> dynamics. Much knowledge on plant tissue O<sub>2</sub> dynamics has been gained due to ongoing development of new O<sub>2</sub> measuring techniques. In the following, we therefore present an overview of the progress in determining plant tissue O<sub>2</sub> status. We then use this overview to identify future opportunities to obtain further insight of plant O<sub>2</sub> dynamics.

### Measuring O<sub>2</sub> in plant tissues: past, present, and future approaches

The earliest measurements of O<sub>2</sub> in plant tissues made use of various forms of gas analysers. Gases sampled from tissues using syringes (or by crushing in mercury) was drawn into

a glass chamber, which was then brought into contact with an O<sub>2</sub> absorbing solution, allowing for the decrease in gas volume to be monitored (Thoday 1913; Scholander 1947). The earliest methods to our knowledge was the Bonnier-Mangin microtechnique (Thoday 1913) with adaptations by Scholander (1947), used as late as 1994 on tomato (*Solanum lycopersicum*) and potato tubers (Nery and Calbo 1994). Other studies (Laing 1940) applied the Henderson-Haldane gas analyser, widely used in human physiology research, for measuring O<sub>2</sub> in aquatic plants. Finally, gas sampled from tissues with syringes has been analysed using gas chromatographs (Spalding *et al.* 1979; Tjepkema and Cartica 1982). To our knowledge, the results obtained using gas analysers have not been subject to a direct comparison with other methods such as microsensors.

The development of microsensors allowed for O<sub>2</sub> measurements with improved spatial and temporal resolution (Revsbech 1989). The most popular sensors are miniaturised, stiff and narrow (down to 3  $\mu\text{m}$  tip diameter) Clark-type sensors, ideal for penetrating plant tissue and measuring O<sub>2</sub> within plants (Pedersen *et al.* 2020). Optical O<sub>2</sub> sensors (O<sub>2</sub> optodes) can also be positioned within plant tissues, especially if built into a syringe. Foils coated with O<sub>2</sub>-sensitive fluorescent dyes and optical O<sub>2</sub> sensor nanoparticles are often used for mapping O<sub>2</sub> on plant surfaces and their surroundings (Rudolph *et al.* 2012; Brodersen *et al.* 2014; Koren *et al.* 2015). However,

optical O<sub>2</sub> sensor nanoparticles have only seen limited application inside plants tissues due to their size of up to 600 nm (Schmäzlin *et al.* 2005; Shaw and Honeychurch 2022). Because of microsensor popularity in plant research, >70% of the 1567 observations of plant tissue O<sub>2</sub> used in this study were obtained using an O<sub>2</sub> microsensor. For a more detailed review on use of microsensors in plant biology, see Pedersen *et al.* (2020).

While the abovementioned methods allow for direct O<sub>2</sub> measurements within plant tissues, more indirect methods have also been applied. Measurements of radial O<sub>2</sub> loss from roots using cylindrical platinum electrodes have been used to model root internal O<sub>2</sub> status (Armstrong and Wright 1975); see also Jiménez *et al.* (2021) for a recent review on methods on measurement of radial O<sub>2</sub> loss from roots. Another indirect measurement of tissue O<sub>2</sub> status is related to the expression of genes strongly upregulated upon low O<sub>2</sub> (referred to as hypoxic reporters) (Mustroph *et al.* 2010).

In the present meta-analysis, we identified 'tissue type', 'submergence', 'light' and 'light × submergence' interaction as important environmental factors for plant O<sub>2</sub> status. When conducting meta-analysis, researchers should consider the risk of 'publication bias', a term originating from health sciences describing information suppression mechanisms (Dickersin 2005). This includes language bias or accession bias (selective inclusion of studies that are accessible, and published in English), and outcome bias (selective reporting of statistically significant results) among others (Dickersin 2005). The latter would translate to studies reporting surprisingly low or high plant tissue O<sub>2</sub> levels, or significant treatment effects of for example plant submergence, being more likely to reach publication than negative results, either due to bias at the investigator or editorial level. The concern that O<sub>2</sub> measurements of experiments revealing no treatment effects are under-reported cannot be excluded. However, considering the large number of studies across time, space, species, and methodology describing significant effects of light and submergence on plant tissue O<sub>2</sub> levels, we consider their potential importance as validated by this study. We also believe that our data gives an impression of the range and variability in plant tissue O<sub>2</sub> levels, although random sampling both at community, species and tissue level would be required to achieve a distribution resembling the 'true' distribution of O<sub>2</sub> in all plants.

Methods to conduct non-invasive O<sub>2</sub> measurements at high spatial and temporal resolution should promote expansion of plant tissue O<sub>2</sub> research from predominantly studying the effect of submergence into other areas. Areas already under investigation include studying plant O<sub>2</sub> levels in relation to plant development (Weits *et al.* 2019), pathology (Kumari *et al.* 2017) and plant signalling (Weits *et al.* 2021). It has been noted that unravelling tissue O<sub>2</sub> dynamics in plants still lacks behind the mammalian field (Ast *et al.* 2012; Schmidt *et al.* 2018). This is underlined by the search term 'tissue hypoxia' in the Web of Science BIOSIS Previews database

resulting in 34 970 records belonging to the 'Mammalia' taxa compared with 1041 in 'Plantae' (performed in November 2022). In mammals, cancer research resulted in the development of methods such as positron emission tomography (PET), genetically encoded sensors, and <sup>18</sup>F NMR for non-invasive O<sub>2</sub> measurements (Schmidt *et al.* 2018). Challenges in translating these methods to plant biology include plant tissue autofluorescence (Ast *et al.* 2012). However, PET scans of plants tissues used to study carbon dynamics illustrate the possible future application of this technique in plant science (Mincke *et al.* 2021).

## Conclusion

Based on the extraction of 1567 recorded tissue O<sub>2</sub> levels from 112 plant species, we found that plant tissue O<sub>2</sub> status differs significantly between tissue types. In general, average O<sub>2</sub> status is lower in non-photosynthetic and belowground tissues such as roots (9.8 kPa) and rhizomes (9.4 kPa), compared with the leaf lamina (16.8 kPa) and stems (14.9 kPa). However, exceptions exist as exemplified by photosynthesising aquatic roots and mangrove pneumatophores showing relatively high O<sub>2</sub> status compared with other root tissues. Environmental conditions that significantly affected plant O<sub>2</sub> status were light conditions and submergence. Interestingly, light interacted significantly with submergence on plant tissue O<sub>2</sub> status so that while submergence under light caused shoot O<sub>2</sub> status to increase by 44% compared with non-submerged shoots, submergence in the dark reduced shoot tissue O<sub>2</sub> by 42%. Thus, restricted gas exchange during submergence in light caused shoot tissues to accumulate photosynthetic O<sub>2</sub>, while the O<sub>2</sub> status declined towards hypoxic conditions upon submergence in the dark. Tissue O<sub>2</sub> under submergence correlated stronger with floodwater O<sub>2</sub> in dark than in light, where shoot tissues could photosynthesise and thus render plant tissues less dependent on floodwater O<sub>2</sub>. While the development of O<sub>2</sub> microsensors has resulted in increased insight into plant tissue O<sub>2</sub> dynamics, the field lacks behind the knowledge of O<sub>2</sub> dynamics in mammalian tissues. We therefore predict that the use of non-invasive techniques in the future will shed further light on plant tissue O<sub>2</sub> dynamics.

## Supplementary material

Supplementary material is available [online](#).

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**Author affiliations**

<sup>A</sup>The Freshwater Biological Laboratory, Department of Biology, University of Copenhagen, Universitetsparken 4, 3rd Floor, Copenhagen 2100, Denmark.

<sup>B</sup>Department of Food, Agricultural, Environmental and Animal Sciences, University of Udine, via delle Scienze 206, Udine, Italy.

<sup>C</sup>School of Agriculture and Environment, The University of Western Australia, 35 Stirling Highway, Perth, WA 6009, Australia.