Chapter 18 - Waterlogging and submergence

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Flooding, resulting in soil waterlogging and in many situations even complete submergence of plants, is an important abiotic stress in many regions worldwide. The number of floods has increased in recent decades (Figure 18.1), and the severity of floods is expected to increase further in many regions of the world.

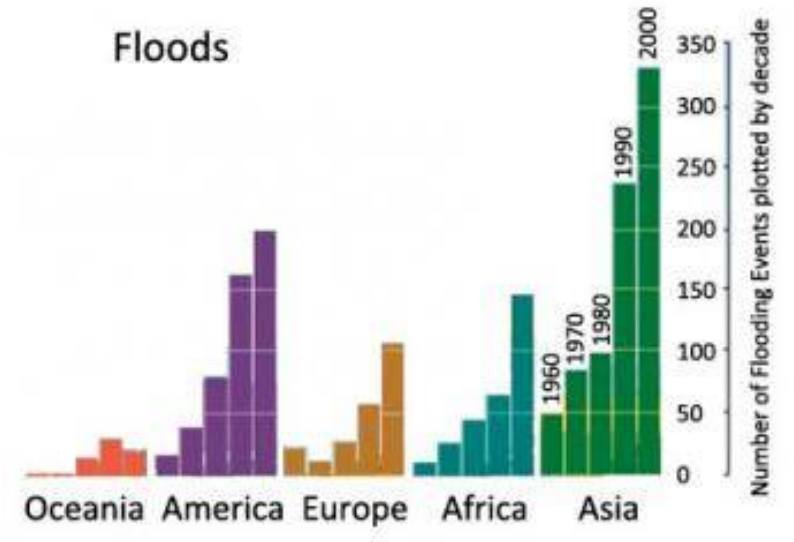


Figure 18.1 Flooding events in various regions of the world. Graphs show the number of floods classified as a disaster in the International Disaster Database of the University of Louvain, Belgium, for decades from 1950 through 2000. Floods resulted from rivers after heavy rainfall or snow melts, and coastal flooding. Data from the Millennium Ecosystem Assessment map (http://maps.grida.no/go/graphic/number-of-flood-events-by-continent-and-decade-since-1950)

Flooding reduces agricultural production, and floods shape many natural plant communities (e.g. floodplains, wetlands, salt marshes). A spectacular example of an important natural ecosystem shaped by flooding is the Amazon Floodplain forests, in which seasonal floods are deep and prolonged (Figure 18.2).



Figure 18.2 Amazon Floodplain forests. Várzea forest during flooding season at Solimões River near Manaus-AM, Brasil. (Photographs courtesy B. C. Arenque Musa).

Tolerance of plants to soil waterlogging, and to shoot submergence, varies greatly; ranging from many very sensitive 'dryland' species (including most of our crops) to highly tolerant species such as rice and other wetland species. In addition, aquatic and marine plant species have adopted submerged lifestyles under water. Knowledge of tolerance mechanisms will underpin future breeding of more robust crops, and understanding plant responses to flooding will aid management of plant communities in flood-prone environments. Recent breakthroughs in submergence tolerance research on rice resulting in new varieties will help sustain a growing world population (see Case Study 1), and has improved knowledge of plant adaptive mechanisms to flooding stress.

This chapter summarises the adverse conditions faced by plants when water is in excess, and acclimations and adaptations to flooding stress. We consider the situations for roots in waterlogged soils, and for shoots submerged by overland floods. Four case studies highlight important developments in plant flooding research. The chapter demonstrates that interdisciplinary research in plant sciences has improved knowledge of plant flooding tolerance, with applications in crop breeding.

18.1 - Soil aeration, redox chemistry, soil toxins and changes in nutrients

In drained soils, diffusion in the gas phase of the bulk soil sustains the O_2 supply needed for roots to respire at optimal rates. Soil flooding impedes O_2 movement into soils, and so roots experience hypoxia (sub-optimal O_2) and anoxia (absence of O_2). O_2 is the terminal electron acceptor of mitochondrial electron transport, so anoxia inhibits respiration and the resulting energy deficit has major implications for roots. In addition, decreases in soil redox potential result in significant changes to the soil elemental profile. The sequence of events following soil flooding are listed in Table 18.1, which also shows the rates of change measured in two soil types differing in temperature and organic matter contents. The impeded gas exchange during soil waterlogging leads to root hypoxia or anoxia, high CO_2 in the root zone, and phytotoxins in reduced soils, all with consequences for root metabolism, nutrient acquisition, and growth of roots and shoots.

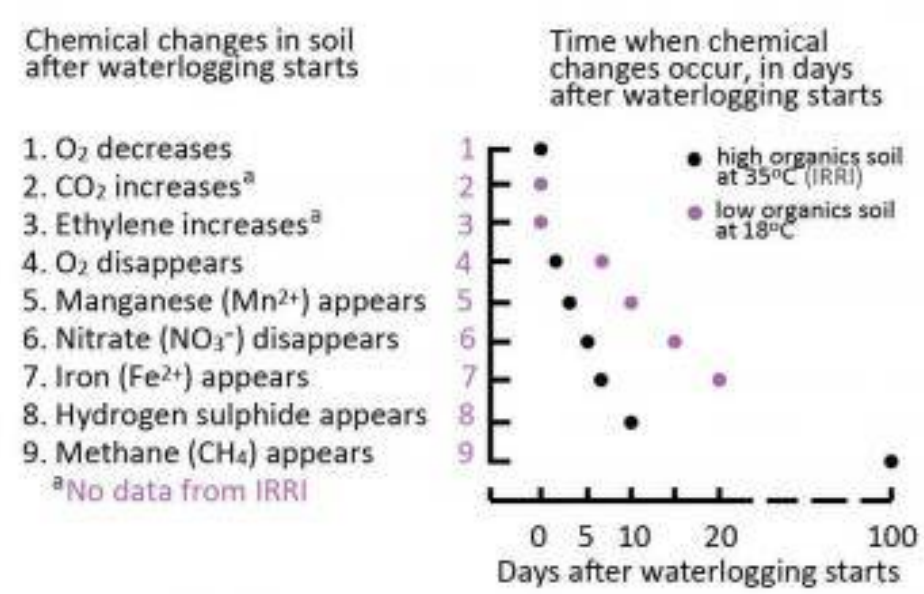


Table 18.1 List of chemical changes in two soils and a linked graph of when they occurred during 100 days of waterlogging. One soil is a sandy loam containing little organic matter at 18°C from Muresk, Western Australia, the other a clay soil high in organic matter at 35°C from the International Rice Research Institute (IRRI) in the Philippines. Based on Setter and Belford (1990).

As soon as O_2 is depleted, NO_3^- is used by some soil microorganisms as an alternative electron acceptor in their respiration; NO_3^- is reduced to NH_4^+, so it becomes the main form of mineral nitrogen in waterlogged soils. In the rhizosphere of roots with radial O_2 loss (ROL), however, NH_4^+ can be converted back to NO_3^-, with both these forms of mineral nitrogen absorbed by roots. Manganese oxides are the next electron acceptors used by anaerobic microorganisms, followed by iron oxides resulting, respectively, in elevated concentrations of Mn^{2+} and Fe^{2+} in the soil solution; these soluble forms often increase to levels that are toxic to plants. Further decrease in the redox potential results in the reduction of SO_4^{2-} to H_2S, which is also potentially toxic. In addition to these inorganic phytotoxins (Fe^{2+}, Mn^{2+}, or H_2S), various short-chain fatty acids can also accumulate in waterlogged soils. In addition to phytotoxins, some nutrients change in availability in flooded soils; e.g., P becomes more available, whereas Zn becomes less available (reviewed in Ismail et al., 2007).

High concentrations of both Mn^{2+} and Fe^{2+} are considered to be major constraints for growing sensitive cultivars of wheat in waterlogging-prone areas of Australia (Khabaz-Saberi et al. 2010); these elemental toxicities also limit rice yields in many flooded areas around the globe. Also detrimental to plants is the accumulation of metabolites (e.g. acetic acid, butyric acid, propionic acid) produced as a result of anaerobic metabolism by microorganisms in waterlogged soils. The types and amounts of these organic compounds depends upon the fermentative character of the microorganisms, the organic matter in the soil, and on soil conditions such as pH and temperature. These compounds can have adverse effects on root growth (e.g. cell division and viability) and nutrient acquisition (e.g. activity of various membrane transporters, membrane permeability) and, ultimately, shoot growth (Shabala 2011).

Of particular interest is the finding that the function of root plasma membrane transporters may be affected by these phytotoxins or secondary metabolites in waterlogged soils. Transporters located at the root-rhizosphere interface would be exposed to these toxins in waterlogged soils. Ion flux kinetics for plant roots changed rapidly upon exposure to secondary metabolites (Pang et al. 2007), and uptake of phytotoxins *per se* may be mediated by membrane transporters. Whether wetland plant roots, as compared with waterlogging-sensitive crops, posses membrane transporters more resistant to these toxins is an important question for future research, with possible implications for improving waterlogging tolerance in crops.

Case Study 18.1: Rice ecotypes and systems

Abdelbagi M. Ismail, International Rice Research Institute, The Philippines

The genus *Oryza* constitutes about 24 species, 20 of which are wild and only *O. sativa* and *O. glaberrima* are cultivated. *O. sativa* is grown worldwide, whereas *O. glaberrima* is restricted mostly to West Africa. Ecological and geographic distribution of these species is largely determined by temperature and water availability. *O. sativa* is cultivated on about 144 million ha worldwide, from 50° N in North China to 35° S in Australia (New South Wales) and in Argentina. It is also grown from 3 m below sea level in Kerala, India, to as high as 3000 m in Nepal and Bhutan. Two broad categories are generally identified within *O. sativa*, with some overlaps; japonica varieties are mostly grown in temperate regions, while indica varieties are grown in tropical and subtropical areas. A third category— tropical-japonica — is mostly grown in the uplands of the tropics and subtropics. Japonica types are known for their better tolerance of low temperatures compared with indica types, and japonica types also have shorter, thicker grains that are softer and stickier when cooked.

Rice is grown on a variety of soils, but the physical ability of the soil to hold water is an important property, so medium- and heavier-textured soils are typically favoured over light-textured sandy soils. It is also grown under variable water regimes and hydrological conditions, from aerobic soils as in uplands, to flooded soils in irrigated and rainfed lowlands, to long-duration flooded conditions in flood-prone areas (Figure 1).

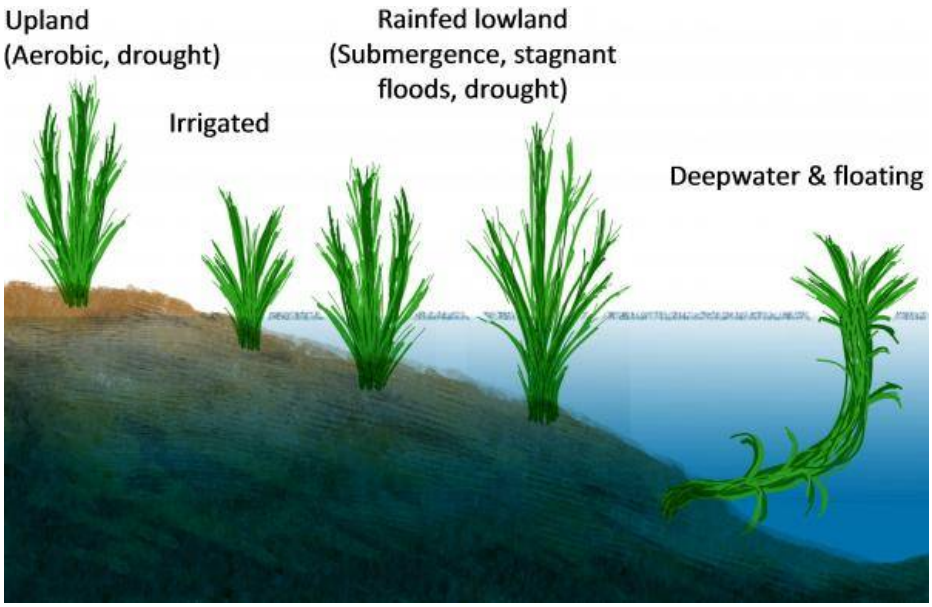


Figure 1. Rice ecologies and ecosystems based on local hydrology. (Diagram courtesy M. A. Ismail)



Figure 2. Rice terraces of Madagascar. A typical example where rice is grown under different ecologies, from uplands at the top of the slope, to more favourable rainfed and irrigated areas midway, to flood prone areas at the bottom of the slope. Farmers normally grow different varieties based on adaptation to each condition. (Photograph courtesy A. M. Ismail)

The enormous plasticity in rice to adapt to these diverse ecologies led to the development of substantial numbers of rice cultivars with diverse morphology, phenology and other adaptive and grain characteristics. The Genetic Resource Center of the International Rice Research Institute hosts over 117,000 accessions collected worldwide (http://irri.org/our-work/research/genetic-diversity/international-rice-genebank).

This peculiar diversity within *Oryza* species made rice one of the most widely grown crops over an extreme range of habitats, and a spectacular model for plant ecophysiological and genetic studies. Various types of models were used to classify rice types based on field ecologies. The most widely used classification distinguishes four broad categories; upland, irrigated lowland, rainfed lowland and flood-prone ecosystems (Maclean et al., 2002). Characters of varieties suitable for each ecology are mostly determined by local hydrology, and in some cases multiple systems co-exist based on the toposequence (Figure 2).

Upland rice is grown in aerobic unbunded soils with topographies ranging from undulating and steep sloping lands with high runoff, to low-laying valleys and well-drained flat lands. Soils vary considerably in texture, fertility and water holding capacity; from poor highly leached soils of West Africa, to fertile soils in Southeast Asia. About 13% of the world rice is grown in uplands, but with low yields of about 1 t ha^{-1}, and farmers are among the poorest. Upland varieties are mostly short maturing, with deeper roots (drought avoidance) and with higher tolerance of acid soils.

Irrigated ecosystem is the largest rice production system, covering 55% of the world rice area and producing over 75% of world rice grains. Fields have assured water supply and rice is grown in puddled soil in bunded fields with water depths of 2.5-10 cm through most of the season, and with 1-3 crops per year depending on location and farming systems. Dwarf high yielding varieties that are responsive to high use of fertilizers are predominant, and yields are usually high, averaging over 5 t ha^{-1}.

Rainfed lowlands constitute about one quarter of rice world lands and contribute about 18% of rice production. These areas are generally densely populated with poor communities, and are prone to both drought and submergence because of lack of water control, besides adverse soils, inhibiting adoption of high-yielding varieties and use of high-cost fertilizer inputs. Local landraces with yields of less than 2 t ha^{-1} still dominate in most areas; however, new high-yielding varieties tolerant of prevailing abiotic factors are becoming available over recent years and are gradually replacing existing local landraces.

Flood-prone rice ecosystems are subjected to uncontrolled floods, ranging from transient flash-floods causing complete submergence, to longer term floods of 0.5 m to over 4.0 m for most of the season, and sometimes associated with excess salinity, acid sulfates and drought. Over 15 million ha in South and Southeast Asia are annually affected by uncontrolled floods. Yields are low, averaging 1.5 t ha^{-1}, and yet these areas support over 100 million people. Traditional varieties still dominate because they are better adapted to water fluctuations than modern varieties. Recently, varieties that tolerate complete submergence are becoming available through the incorporation of the *SUB1A* gene (see also main text). These varieties tolerate 1-2 weeks of complete submergence and considerable yield benefits have been achieved in farmers' fields, with yield advantages of 1 to over 3 t ha^{-1} (Mackill et al., 2012).

The extreme diversity in adaptation to various ecological and hydraulic conditions made rice one of the most widely grown cereal crops worldwide; and an interesting model for crop improvement research. Currently, rice is the most important food crop in developing word and the stable food for over half of the world population.

Further reading on this topic:

Maclean JL, Dawe DC, Hardy B, Hettel GP (2002) Rice Almanac. Los Banos (Philippines): International Rice Research Institute, pp 16-24 http://books.irri.org/0851996361_content.pdf

Mackill DJ, Ismail AM, Singh US, Labios RV, Paris TR (2012) Development and rapid adoption of submergence-tolerant (Sub1) rice varieties. Adv Agron **115**: 303-356.

18.2 - Water chemistry of floods causing complete submergence

The much slower diffusion of gases in water, compared with in air (Table 18.2), presents a challenge to plants that become submerged.

Table 18.2

Diffusion coefficients ($m^2 s^{-1}$) of O_2 and CO_2 in air and in water, at different temperatures

Temperature (°C)	Air (x 10^{-5})		Water (x 10^{-9})	
	O_2	CO_2	O_2	CO_2
0	1.79	1.39	1.10	1.15
10	1.91	1.49	1.57	1.46
20	2.03	1.60	2.10	1.77
30	2.15	1.70	2.75	2.08
40	2.27	1.81	3.93	2.42

Data from Armstrong (1979) and Sand-Jensen (2004)

Gas exchange of submerged plants is greatly impeded by their environment. Although the distances across diffusive boundary layers (DBL) around leaves are the same order of magnitude in water and air, the 10,000-times slower diffusion in water results in the high resistance to gas exchange across the DBL in water. Consequently, submerged aquatic plants have developed adaptive features of their leaves that reduce the DBL, to facilitate exchange of O_2 and CO_2 with the surrounding water (Table 18.3).

Table 18.3

Comparison of leaf traits influencing gas exchange and photosynthesis by terrestrial wetland plants when under water and submerged aquatic plants

Leaf traits for:	Terrestrial wetland plants	Submerged aquatic plants
Morphology		
Leaf size	Medium-large	Small-medium
Dissected/lobed	Rare	Common
Strap-shaped	Rare	Common
Leaf thickness	Moderate-thick	Thin
Hairs/trichomes	Rare	Absent
Surface hydrophobicity resulting in leaf gas films	Common	Absent
Anatomy		
Stomata	Always present	Absent/non-functional
Cuticle	Always present	Absent/highly reduced
Chloroplasts in epidermal cells	Only in guard cells	Common
Aerenchyma	Variable	Variable
Supporting fibres	Always present	Rare
Porosity of lamina	High in thick, low in thin lamina	High in thick, low in thin lamina
Photosynthetic pathway		
C3	Common	Common
C4	Rare	Rare (but uncertain)
CAM	Absent	Rare
*Ability to utilise HCO_3^-	Absent	Common

*Use of HCO_3^- can involve external conversion to CO_2 owing to low pH within the diffusive boundary layer and/or the enzyme carbonic anhydrase (CA) at the cell/tissue surfaces, or via the uptake of HCO_3^- and its intracellular conversion to CO_2 by CA. The ability to utilize HCO_3^- is common in algae, macroalgae and aquatic angiosperms (summarised by Pedersen et al. 2013).

Other leaf features/properties can also differ between terrestrial wetland plants and submerged aquatic plants, such as: venation, lignification, stiffness, surface topography, differences between adaxial and abaxial surfaces, and in the case of some halophytic wetland species presence of salt bladders and glands (Table 18.3).

In addition to the slow diffusion, the solubility of O_2 in water is poor. One litre of air contains approximately 33-fold more O_2 than one litre of water at 20 °C at sea level (101 kPa). Temperature affects the solubility of O_2; the solubility decreases with increasing temperature (Figure 18.3). Imagine a kettle that heats up; the water starts bubbling long before it reaches the boiling point because the solubility of gaseous N_2 and O_2 steeply decreases as the temperature rises.

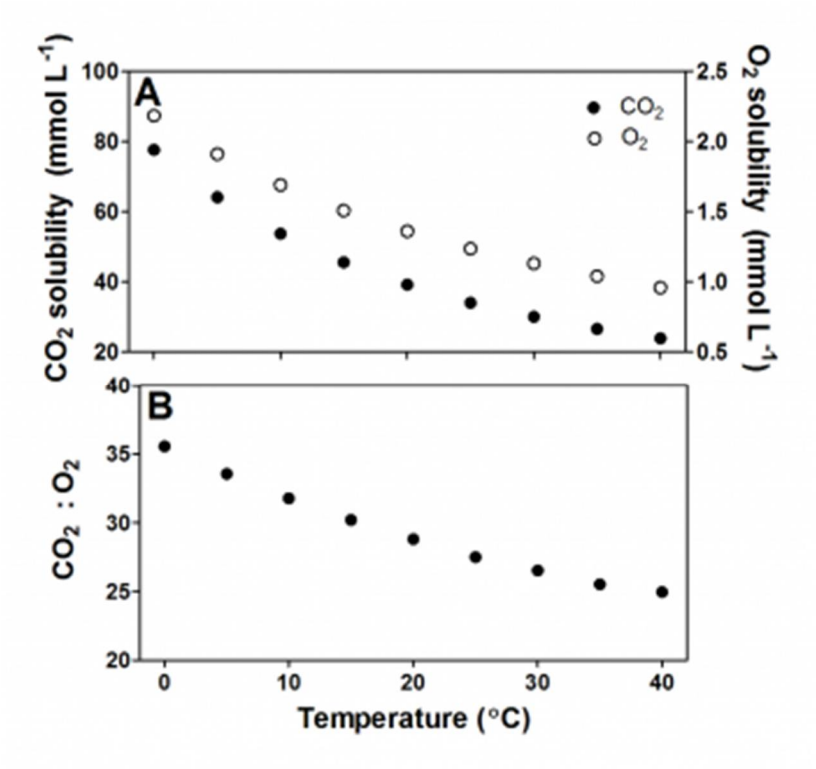


Figure 18.3. Solubility of pure CO_2 and O_2 in water (A) and the ratio of CO_2:O_2 solubility *versus* temperature (B). The solubility of CO_2 at 20°C is ~ 29-fold higher than the solubility of O_2, and when temperature rises the solubility of CO_2 decreases faster than for O_2. Data for O2 from Himmelblau and Arends (1959); for CO_2 from Wilhelm, Battino and Wilcock (1977).

Salinity also affects the solubility of O_2 in water. Sea water contains 35 ppt (parts per thousand) salt, which is approximately 550 mM NaCl, and at 20 °C contains only ~231 µmol O_2 L^{-1} as compared to freshwater that holds ~290 µmol O_2 L^{-1}.

Like for O_2, the solubility of CO_2 also decreases with increasing temperature and salinity. However, the chemistry of CO_2 in water is more complicated than for O_2, as CO_2 reacts with water in the following pH-dependent equilibria (Figure 18.3 and its caption). CO_2 reacts with water (H_2O) and forms carbonic acid (H_2CO_3). However, H_2CO_3 dissociates immediately into a proton (H^+) and bicarbonate (HCO_3^-) so the dissolution of CO_2 into water causes pH to drop. At high pH, HCO_3^- can further dissociate into a second H^+ and carbonate (CO_3^{2-}). The sum of CO_2, (H_2CO_3), HCO_3^- and CO_3^{2-} is referred to as dissolved inorganic carbon (DIC) and the relative distribution of the three main forms of inorganic carbon is determined by pH (Figure 18.4).

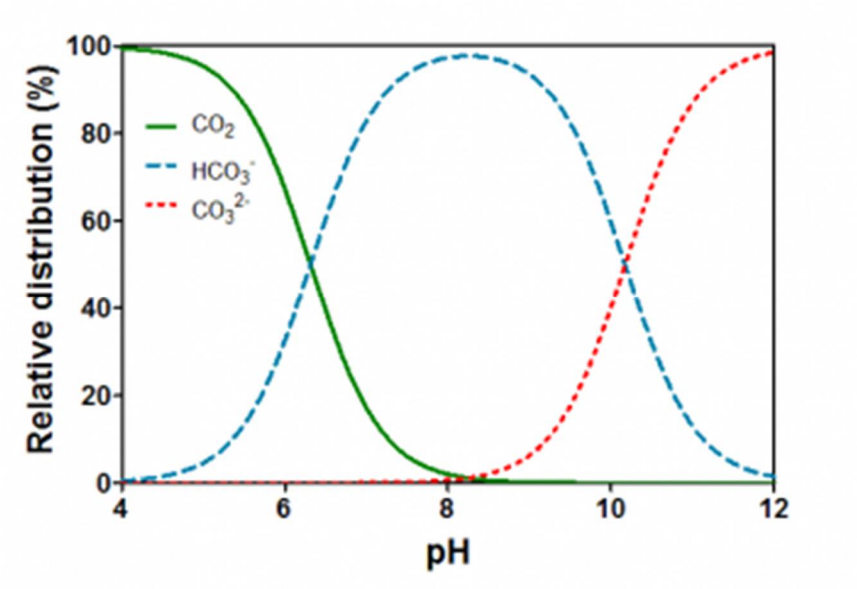


Figure 18.4. Relative distributions of CO_2, HCO_3^- and CO_3^{2-} as a function of pH in water. When CO_2 dissolves in water, it reacts with water: $CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + H^+$. The apparent pKa_1 is 6.532; only a little CO_2 is converted into carbonic acid while the majority remains as CO_2(aq). pKa_2 is 10.329. So, water pH influences the availability of CO_2 to submerged plants. Below pH 6 most of the dissolved inorganic carbon is available as CO_2. Between pH 7 and 10, the alkaline ion HCO_3^- dominates, which can be used only by the most specialised aquatic plants as an inorganic carbon source. Only at pH higher than 10, a significant proportion of the dissolved inorganic carbon is in the form of CO_3^{2-}, which plants cannot use in photosynthesis. Stumm and Morgan (1996).

The concentrations of O_2 and CO_2 can differ markedly between water bodies. In net heterotrophic systems the waters contain dissolved CO_2 concentrations above that when in equilibrium with air; respiration by organisms consuming labile carbon compounds results in depletion of O_2 but enrichment of CO_2. By contrast, in net autotrophic systems, photosynthesis exceeds respiration and so depletes CO_2 and elevates O_2 in these waters. So, O_2 concentrations in floodwaters can range from severely hypoxic (net heterotrophic) to above air equilibrium (net autotrophic); and both these conditions can occur in diel patterns (respiration only during night; photosynthesis dominates during day), for example as measured in fields of submerged rice.

Besides the slow diffusion of CO_2 in water, photosynthesis by submerged plants can also be limited by light. In water, light attenuation follows an exponential function,

$$I = I_0 \, e^{-z\varepsilon}$$

where I is the available light at a given depth (z), I_0 is the light level at the surface, ε is the extinction coefficient, and e is the exponent. The extinction coefficient of water itself is 0.03 m^{-1} so in ultra clear water, rooted plants could grow down to a depth of 75 m where 10% of the surface light would still be present, which happens to be the approximate depth limit of seagrasses). However, dissolved coloured organic carbon (e.g. humic acids), chlorophyll in planktonic algae, and particles suspended in the water (e.g. silt), all reduce light penetration in floodwaters. In turbid floodwaters, light attenuation can be as much as 90% in the upper 10 cm, whereas more typical depths for a 90% reduction of light in floodwaters might be between 0.5 - 2 m.

Case Study 18.2: Rice coleoptiles - an excellent model for studies on oxygen deprivation

Brian Atwell, Macquarie University, Australia

Flooding is an environmental hazard writ large across the agricultural regions of the planet. Major inundations constrain food production because few of the world's crop species are hydrophytes (wetland plants). It is essential to discover mechanisms of tolerance that can be used as a basis for breeding and engineering flood tolerance in rice and also other crops.

Rice (*Oryza* spp.) is notable among the staple crops of the world for its extreme flood tolerance. Thus it has been a natural choice for discovery of flood-tolerance mechanisms. More specifically, the coleoptile of rice is subject in nature to extreme hypoxia or even anoxia and over thousands of years these organs have concentrated gene expression patterns that confer tolerance to O_2 deficits.

The coleoptile, a small sheath that emerges from the seed (caryopsis) as a 'proto-shoot', is especially critical in this scenario because it grows preferentially while roots and leaves remain suppressed without O_2 present. Coleoptiles also have special characteristics to make their growth energetically 'cheap'. They consist of pre-formed cells and can increase in length by 30% each hour (Figure 1 of this Case Study) while the organ as a whole can elongate by 3 cm in a day under water. This illustrates that coleoptiles have evolved to be a perfect emergent organ, providing a conduit from anoxic soil/hypoxic floodwater to the water surface above. Reaching O_2 is essential before energy deficits overwhelm the embryonic meristems. Indeed, this specific role of coleoptiles was called a 'snorkel' almost 50 years ago (Kordan, 1974) because it was realised that on reaching a source of O_2, other organs like leaves and roots could grow.

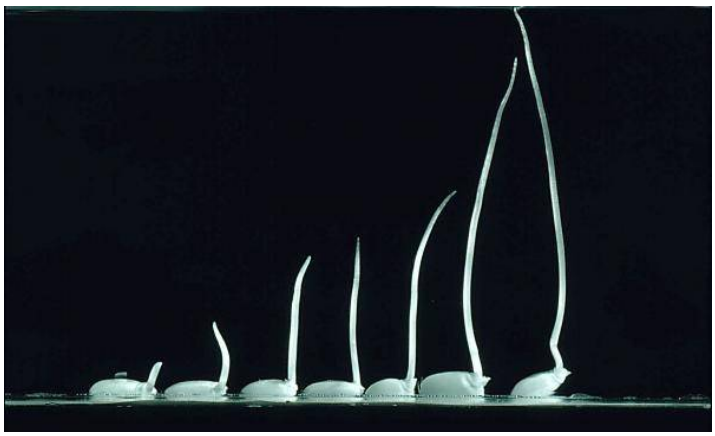


Figure 1. A time series of rice seedlings growing in hypoxic stagnant solution for 6 days. Note the lack of roots or true leaf. The seedling on the far right has commenced a new phase of development, with the production of a mesocotyl at the base of the coleoptile. This coleoptilar node is the first true shoot meristem. (Photograph courtesy of R. Oldfield and B.J. Atwell).

Fast coleoptile growth under water is stimulated by ethylene. The buildup of this phytohormone causes coleoptiles of submergence-tolerant genotypes to 'stretch' towards to the water surface. Hence, rice coleoptiles constitute an example of the 'escape' strategy. As described in Case Study 1, a *failure* to perceive ethylene in shoots ensures survival in mature plants under long-term floods; the presence of the *Sub1A* gene induces dormancy and conserves carbohydrates. By contrast with that quiescent strategy, in areas such as river deltas with prolonged deep floods, internode elongation is beneficial for escape and with the discovery of two snorkel genes, has also been shown to be an ethylene-mediated phenomenon.

Mechanisms of anoxia tolerance in plants have been elucidated through studies of coleoptiles. Ethanolic fermentation can accelerate in anoxia because the Pasteur Effect speeds up glycolysis, using carbohydrates from the seed reserves. This provides a modestly better ATP supply than a non-hydrophyte could generate. Indeed, while ethanol was long been thought to be the dominant player in fermentation, other pathways involving haemoglobin and nitric oxide are now being invoked in energy production under anoxia. The realisation that maintenance and even growth in anoxia could be achieved by preserving critical energetically demanding reactions at the cost of non-essential reactions has opened new avenues of research. For example, we now know that protein synthesis becomes a very dominant use of ATP in anoxia while many other energised reactions succumb to energy shortages. Modification of the hierarchy of energy use occurs in animals and could be an evolutionary step unique to hydrophytic plants.

Ultimately, membrane potentials are vital for all living cells. The cost of maintaining potentials has been studied in depth, including under anoxia in coleoptiles. Proton pumping between compartments is vital and in anoxia, ATP-driven proton pumps become disabled. This induces a fall in the vital cytosolic pH but this fall is modest in rice whereas acidification can be lethal in intolerant species. Most remarkably, pyrophosphate is an alternative energy source to ATP for pumping protons in plants. This relatively 'cheap' energy source appears to energise proton pumping into the cell vacuole by a unique protein that is expressed most strongly in anoxia-tolerant cultivars of rice and is inducible within < 2 h of imposition of anoxia. Genetically knocking out the gene for this tonoplastic proton pump increases sensitivity to anoxia. Thus, genes encoding a pyrophosphate-driven pump at the tonoplast have been identified as targets for improving survival during flooding and have also recently been implicated in regulation of Na^+ compartmentation crucial also to salinity tolerance.

This short account of some of the applications of rice coleoptiles to improve our understanding of stress tolerance illustrates the power of an ideal model. That one simple, undifferentiated organ could instruct us about hormone physiology, energy metabolism and membrane integrity is truly remarkable. This does not preclude the need for studies in more organisms, including wild plants or animals, but does reinforce the importance of model systems

Further reading:

Kordan HA (1974) The rice shoot in relation to oxygen supply and root growth in seedlings germinating under water. New Phytol **73**: 695–697

Edwards JM, Roberts TH, Atwell BJ (2012) Quantifying ATP turnover in anoxic coleoptiles of rice (*Oryza sativa*) demonstrates preferential allocation of energy to protein synthesis. J Exp Bot **63**: 4389-4402

18.3 - Biochemical and metabolic adaptations

Plant adaptation to O_2-deficient waterlogged soils and flood-prone environments involves a suit of morphological, anatomical, and metabolic traits, as outlined in following sections. Plants need to cope with tissue anoxia, or avoid this adverse condition via a well-developed system of inter-connected gas-filled channels (aerenchyma) for internal O_2 transport to supply submerged parts. Even species with large volumes of aerenchyma can experience anoxia in parts of their body, if only transiently. For these anoxic cells and tissues to survive, acclimative metabolic responses are essential. Furthermore, in addition to O_2 deficits, plants must also cope with increased free radicals and reduced uptake of nutrients as additional components of flooding stress.

18.3.1 - Root respiration and anaerobic metabolism

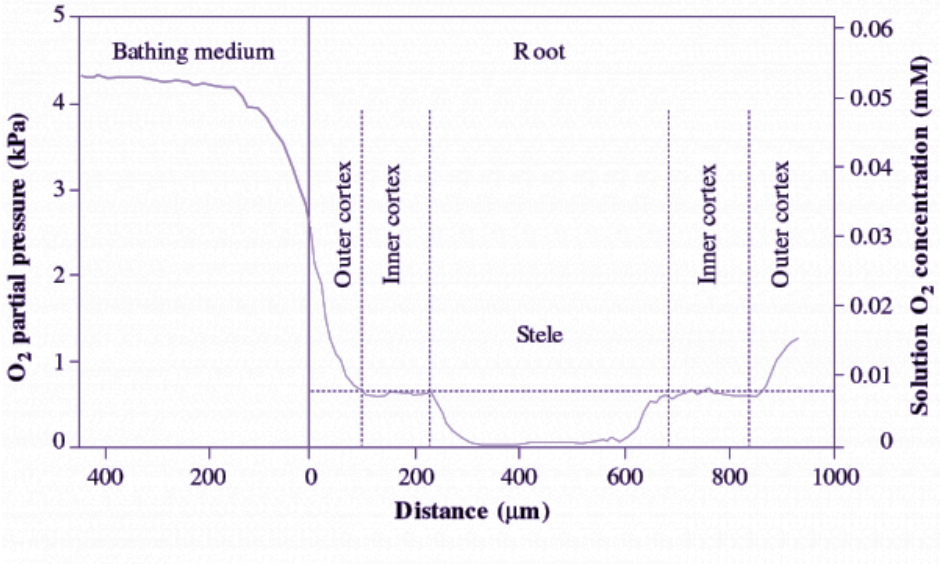


Figure 18.5. O_2 concentration (mM) measured across a maize seedling root by using an O_2-microelectrode. The profile was taken radially through differentiated tissues 75 mm behind the apex of a 135 mm long root. O_2 concentration in the bathing medium was about 0.05 mM (hypoxia), so that the cortex received O_2 whereas the stele had an 'anoxic core'. The abrupt gradient in O_2 status results from the lower porosity and higher metabolic demand in steal tissue as compared with the cortex. (Profile reproduced from Gibbs et al., 1988 with courtesy of W. Armstrong).

When plants are completely submerged and in darkness, so that no photosynthesis occurs, O_2 can become exhausted by respiration resulting in tissue anoxia, especially in tissues/organs buried in anoxic soil. Prolonged anoxia is tolerated by rhizomes, tubers and some shoot organs of wetland species, and by germinating seeds of rice and some paddy weeds (e.g. barnyard grass). More commonly, anoxia can occur in portions of the plant body (e.g. roots), or parts of tissues within roots. 'Anoxic cores', coexistence of an anoxic stele and aerobic cortex, were demonstrated for maize roots in hypoxic conditions, using O_2-microelectrodes (Figure 18.5) and biochemical indicators of fermentative metabolism (Thomson and Greenway 1991).

Roots in drained soil respire by catabolising carbohydrates in the tricarboxylic acid (TCA) cycle, with the 'reducing power' produced used in the electron transport chain (ETC) with O_2 as the terminal electron acceptor. Energy in the form of ATP is generated, predominantly through oxidative phosphorylation in mitochondria (Chapter 2.4). However, in waterlogged soils O_2 is scarce or even absent and therefore respiration is inhibited. Carbohydrates are then broken down via fermentative pathways to yield at least some ATP, produced during substrate-level phosphorylation in glycolysis (Figure 18.6). Conversion of the pyruvate to an end-product, such as ethanol, is essential to remove this metabolite as well as to recycle the NADH to NAD^+, so that the pathway can continue to flow. Breakdown of carbohydrates to ethanol and CO_2 is the principal fermentative pathway in plants. Some lactate and alanine are also produced, but in contrast to fermentation leading to lactate and alanine, ethanolic fermentation can be sustained over days in anoxic tissues, end-product feedback or even toxicity being minimised by leakage of ethanol and CO_2 to the environment.

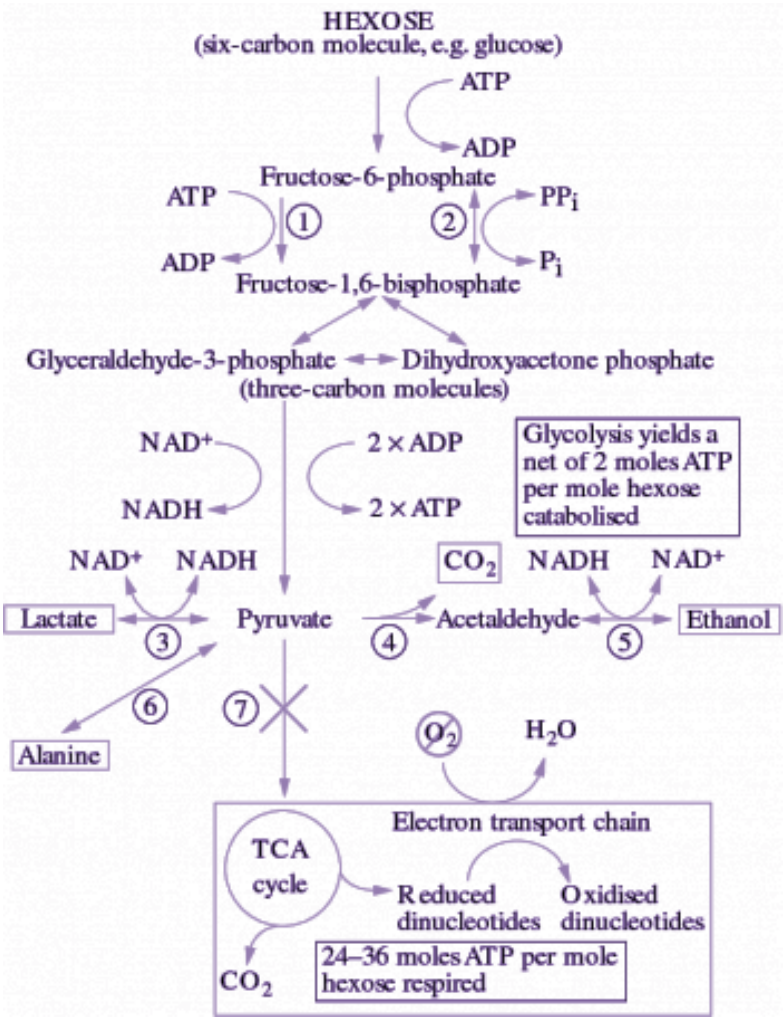


Figure 18.6. Scheme denoting the important metabolic reactions during anaerobic carbohydrate catabolism. Anoxia prevents pyruvate from entering the TCA cycle because O_2 is unavailable as a terminal electron acceptor. Carbon is diverted to fermentative end-products, allowing oxidation of NADH and sustained catabolism of carbohydrates. Key enzymes are: 1. ATP-dependent phosphofructokinase; 2, PPi-dependent phosphofructokinase (PFK); 3, lactate dehydrogenase; 4, pyruvate decarboxylase (PDC); 5, alcohol dehydrogenase (ADH); 6, glutamate-pyruvate transaminase; 7, pyruvate dehydrogenase. The enzyme that catalyses oxidation of NADH as pyruvate is converted to alanine has not been identified. Note that some reactions are reversible (two-way arrows).

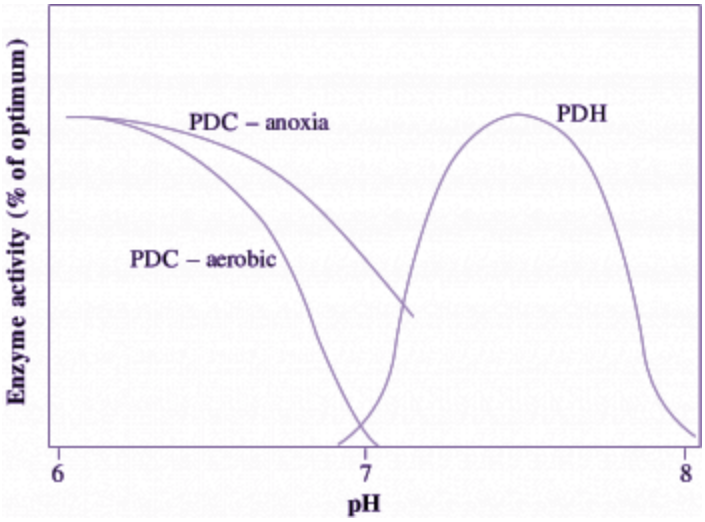


Figure 18.7. Curves showing pH optima of enzymes at the branch point for carbon flow to aerobic and anaerobic pathways. These *in vitro* determinations from extracts of rice coleoptiles indicate how cytoplasmic pH controls carbon flow. In aerobic conditions, pyruvate dehydrogenase (PDH) catalyses entry of pyruvate to the TCA cycle when pH is above 7. Anoxia results in a decline in cytoplasmic pH to below 7, causing PDH activity to give way to pyruvate decarboxylase (PDC) and fermentation to commence (i.e. PDC becomes engaged at pH below 7, whereas PDH ceases to function). In addition, PDC extracted from coleoptiles of rice seedlings previously exposed to anoxia is in a more active state, enhancing pyruvate consumption for ethanol production. Based on Morrell et al. (1989).

Carbon flow from pyruvate to ethanol (with CO_2 also produced) occurs via the fermentative enzymes pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) (Figure 18.6). This flow is probably regulated by the activity of PDC which catalyses the first step of ethanolic fermentation. In wheat roots, for example, the PDC *in vitro* activity approximates the measured *in vivo* rate of ethanol production. Increases in the amounts of PDC and ADH proteins have been observed in a range of plant genotypes and tissues in response to O_2 deprivation. Indeed, these enzymes form part of a suite of 'anaerobic proteins', enzymes synthesised during anoxia. In addition to increased protein abundance, post-translational regulation of PDC activity is also exerted by changes in cytoplasmic pH, which decreases from around 7.5 in aerobic cells to around 6.8–7.2 in anoxic cells. Below pH 7.2, the activity of PDC reaches its optimum. For example, PDC extracted from anoxic rice coleoptiles becomes very active as pH drops below 7 according to the broad pH response curve in Figure 18.7. Following a return to aerobic conditions, cytoplasmic pH increases back to its normal level, the activity of PDC decreases, and carbon then flows again via pyruvate dehydrogenase (PDH) to the TCA cycle, rather than via PDC for fermentation to ethanol.

During anoxia, normal protein synthesis is replaced by the selective transcription and translation of a set of proteins called 'anaerobic proteins'. In maize roots, there are 20–22 of these proteins which include fermentative enzymes (e.g. PDC and ADH), enzymes involved in anaerobic carbohydrate catabolism (e.g. sucrose synthase and enzymes responsible for the reversible breakdown of sucrose) and several glycolytic enzymes (e.g. aldolase). Other 'anaerobic proteins' of maize include superoxide dismutase (SOD), responsible for scavenging O_2-free radicals. 'Anaerobic proteins' are also formed in rice embryos, with a suit of proteins similar to those described for maize but also others - one very interesting additional 'anaerobic protein' in rice is the tonoplast H^+-pyrophosphatase (Carystinos et al. 1995). By maintaining an 'energised' tonoplast capable of ion and solute transport, this enzyme might help stabilise cytoplasmic pH. Use of pyrophosphate (PPi) as an energy source

reduces the dependence of tonoplast ion transport on ATP regeneration to drive the H^+-ATPase. Studies using knockout mutants in rice have further demonstrated the importance of the H^+-pyrophosphatase for anoxia tolerance (see Case Study 2).

Root tissues can acclimate to low O_2 with improved anoxia tolerance, if exposed to hypoxia (low, but not zero O_2) prior to the onset of anoxia. As examples, roots of maize and wheat survive anoxia more than three times longer if exposed first to hypoxia rather than abrupt transfer from aerated solution into anoxia (Table 18.4). The elimination of ADH activity reduced the survival of maize Adh^-

Mutants to almost zero following anoxic shock but allowed recovery following hypoxic pre-treatment (Table 18.4)

Table 18.4

How hypoxic pretreatment affects survival of root tips of intact plants exposed to anoxia at 25° C. Survival was tested by the ability of root tips to elongate upon re-aeration. Maize, and its mutant deficient in the fermentative enzyme ADH, was pretreated in 4% O_2 for 18 h; wheat was pretreated in 1.5% O_2 for 24 h

	Survival in anoxia	
Plant material	**Anoxic shock**	**Following hypoxic pretreatment**
Maize	Less than 24 h	More than 3 d
Maize *Adh1* null	10% at 6 h	70% at 24 h
Wheat	50% at 5 h	50% at 35 h

Sources of data: Wild-type maize (Johnson et al. 1989), *Adh1* null (Johnson et al. 1994) and wheat (Waters et al. 1991)

Metabolic acclimation set in train by hypoxia included changes in gene expression and therefore the protein complement ('proteome') in cells. Hypoxic pretreatment raised activities of the fermentative enzymes PDC and ADH, and resulted in a faster rate of ethanolic fermentation during the subsequent anoxia. How plants sense and initiate signal cascades to invoke these metabolic acclimations is a current topic of debate and could involve sensing of changes in cellular energy charge, cytosolic pH, and/or possibly oxygen (see Case Study 18.3) or other possibilities.

The changes in fermentative enzymes, together with observations that exogenous sugars prolong tissue survival during anoxia, point to carbohydrate catabolism as an important factor in tolerance to anoxia. Even with fermentation operational, however, the anoxic root cells still face an 'energy crisis', as the ATP generated via fermentation is often insufficient even for cell maintenance in some species. Compared with respiration, fermentation produces 85–95% less ATP per hexose unit consumed. So, although such anaerobic energy generation is vital, a rapid rate of fermentation alone does not endow anoxia tolerance. Pea root tips, for example, ferment 45% faster than maize root tips, but survive less than half as long in anoxia. Greenway and Gibbs (2003) have highlighted that in addition to fermentation, other more subtle aspects of energy consumption must also be involved in anoxia tolerance, such as a reduction of energy requirements for cell maintenance and the redirection of energy flow to

essential cellular processes, including maintenance of membrane integrity, regulation of cytoplasmic pH, and synthesis of appropriate ‘anaerobic proteins’.

The key to anoxia tolerance therefore lies in integration of energy production via anaerobic carbohydrate catabolism and energy consumption in reactions essential for survival. Accumulating evidence suggests two modes of tolerance based on slow and rapid rates of fermentation (Greenway and Gibbs, 2003). As one example of the ‘slow fermentation mode’, lettuce seeds appear to survive anoxia by slowing carbohydrate catabolism in anoxia to less than 35% of the rate in air. After 14 d without O_2, lettuce seeds germinate normally (Raymond and Pradet, 1980). Other plant tissues which survive but do not grow in anoxia, produce an initial burst of fermentative activity over 6–24 h before settling to slower fermentation rates. This two-phase pattern presumably provides the higher ATP required as cells acclimate to anoxia, but then the lower rates of fermentation would conserve carbohydrates for long-term survival. To be of adaptive value, this conservation of substrates through slower catabolism must be compatible with the smaller ATP yield available for cell maintenance. Calculations show that, for example, non-growing beetroot tissue in anoxia used 10- to 25-fold less ATP for cell maintenance than aerobic tissues (Zhang and Greenway, 1994).

The coleoptile of rice provides an example of the ‘fast fermentation mode’, this organ grows in anoxia (a second example is the stem of *Potamogeton* spp.). Fast fermentation is sustained by accelerated glycolysis, a phenomenon known as the ‘Pasteur Effect’. However, even in rice, glycolytic rate is only about twice as fast in anoxia as in air (Table 18.5).

Table 18.5

Changes in the rate of carbohydrate catabolism in several plant species in response to anoxia. Data are expressed as a ratio of the carbon flow through glycolysis in anoxia to the rate in air. Carbon flow during anoxia is estimated from the rate of production of end products of carbon catabolism. Carbon flow in air is estimated from the production of CO_2 and an assessment of carbon used in net protein synthesis

Plant tissue	Tissue treatment	C_{anoxia}/C_{air}
Beetroot storage tissue	Slices, after 24 h of anoxia	0.7
Wheat root tips	Terminal 5 mm excised	1
Maize root tips	Terminal 10 mm excised	1.3
Rice coleoptiles	After 48 h of anoxia, excised	1.7

The glycolytic enzyme ATP-dependent phosphofructokinase (PFK), might in addition to PDC, contribute to control of glycolysis (Figure 18.6) and thus fermentation in the coleoptile of rice. Starch breakdown and sugar transport from the endosperm to coleoptile of rice seedlings in anoxia fuels the ethanolic fermentation. For plants without such starch reserves, however, low carbohydrate levels would limit the rate of anaerobic carbohydrate catabolism in tissues that experience anoxia.

Case Study 18.3 - Can plants sense oxygen?

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Plant life relies on light-energy driven fixation of CO_2 into carbohydrates through photosynthesis. These carbohydrates are subsequently used to construct various plant structures and fuel energy production through respiration in non-photosynthetic tissues and even in photosynthetic cells during dark periods. Respiration requires a sufficient supply of O_2. Cells in various organs of submerged terrestrial plants typically suffer from severe shortage of O_2 and thus energy deficits. The extremely slow diffusion rate of O_2 through water to plant organs and cells means that O_2 supply becomes limiting for respiration. Low O_2 conditions during flooding most frequently occur in root tissues as these are surrounded by a soil environment characterized by very low O_2 levels and because of the absence of photosynthetic capacity to generate O_2. Upon low O_2 conditions metabolism shifts from efficient mitochondrial ATP production (O_2 dependent) to inefficient anaerobic substrate-level production of ATP (glycolysis linked to fermentation), as long as sugar substrates are available. Further adjustments involve restrictions in ATP consumption and translational activities so that general protein turnover slows to save energy.

Reliable sensing of O_2 levels would allow rapid acclimation to declining O_2 in flooded plants. It was shown recently that O_2 sensing is achieved by a mechanism in which the N-end rule pathway of protein degradation serves to regulate the low O_2 response in plants (Gibbs et al. 2011; Licausi et al. 2011). Up to now the unraveling of the O_2 sensing machinery was one of the biggest challenges in flooding research. The identification of such a mechanism sheds light on the earliest step in the signaling pathway leading to low O_2 acclimation (Figure 1).

Many genes that are typically associated with low O_2 conditions in plants are regulated by transcription factors belonging to the so-called group VII Ethylene Response Factors (ERFs). Typical for these ERFs in Arabidopsis (5 members) is that they possess a specific N-terminal motif (N-degron). Due to this motif these proteins are post-translationally modified in an O_2-dependent manner via the N-end rule pathway of protein degradation. The N-terminal of Arabidopsis group VII ERFs is composed of a methionine followed by a cysteine as the second residue. The constitutive activity of methionine amino peptidase cleaves these ERF proteins between the methionine and the cysteine, yielding an N-terminal exposed cysteine. In this conformation the cysteine can be oxidized in an O_2-dependent manner. Under normoxic conditions, cysteine is oxidized and an arginine residue is added to the cysteine, catalyzed by an arginyl tRNA transferase (ATE). In this form the ERF protein can be recognized by the E3 ligase PROTEOLYSIS 6 (PRT6), leading to ubiquitination and 26S proteosome-mediated degradation. However, when O_2 is limited (hypoxia or anoxia) as in many organs of flooded plants, degradation of ERFs is inhibited as a consequence of a lack of cysteine oxidation. Under these conditions stable ERFs can function as transcription factors and drive transcription of genes needed in plants to survive in low-O_2 environments. As soon as the plant is re-oxygenated (e.g. upon withdrawal of flood water) the ERFs are again destabilized and the transcription of hypoxia-induced genes is halted.

At least one Arabidopsis ERF, RAP2.12, is sequestered at the plasma membrane, mediated by an interaction with the membrane-bound Acyl CoA binding proteins 1 and 2 (ACBP1/2). This sequestration of RAP2.12 is functional to prevent degradation by the N-end rule pathway under normoxic conditions. Via docking to ACBP1/2, high levels of RAP2.12 can

be maintained even under normoxic conditions, without the risk of being degraded. Upon hypoxia RAP2.12 translocates rapidly to the nucleus to switch on acclimative pathways for low O_2 conditions (Figure 1).

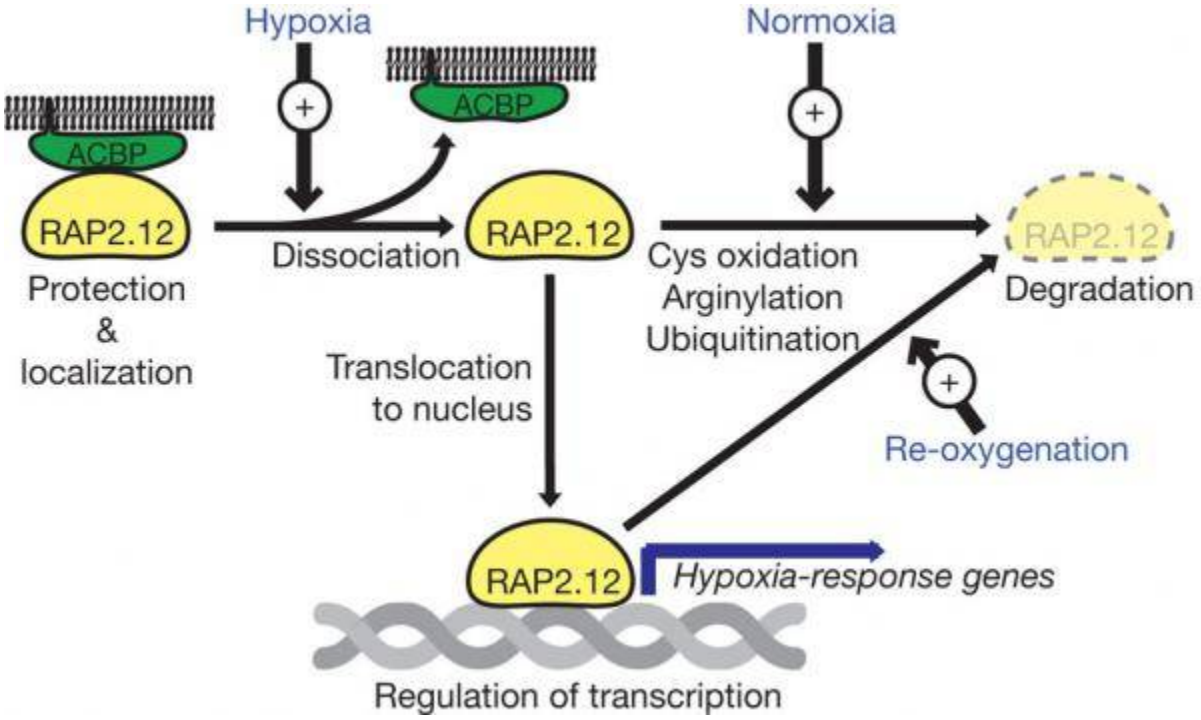


Figure 1. A model describing O_2-sensing in plants. Under normoxic conditions the ERF protein RAP2.12 has a protected plasma membrane localization due to its interaction with Acyl CoA binding proteins (ACBPs). Upon hypoxia RAP2.12 and ACBP dissociate and RAP2.12 moves to the nucleus where it induces transcription of adaptive hypoxia-response genes. Upon re-oxygenation RAP2.12 is rapidly degraded via the N-end rule pathway to down regulate the hypoxia response (Licausi et al. 2011).

Further reading:

Bailey-Serres J, Fukao T, Gibbs DJ et al. (2012) Making sense of low oxygen sensing. Trends Plant Sci **17**: 129-138

Gibbs DJ, Lee SC, Isa NM et al. (2011) Homeostatic response to hypoxia is regulated by the N-end rule pathway in plants. Nature **479**: 415-418

Licausi F, Kosmacz M, Weits DA et al. (2011) Oxygen sensing in plants is mediated by an N-end rule pathway for protein destabilization. Nature **479**: 419-422

18.3.2 - Reactive oxygen species

Hypoxic conditions can favour generation of reactive oxygen species (ROS). The major sources of ROS generation are ETC in mitochondria and oxidase activities (Blokhina et al., 2003). Various ROS species may be produced; among the major ones are superoxide radical ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2). Although H_2O_2 is less reactive than the two other ROS, in the presence of reduced transition metals such as Fe^{2+} (abundant in waterlogged soils), the formation of $OH^{\bullet}$ can occur in the Fenton reaction. These ROS can damage plant cells by causing lipid peroxidation in membranes, DNA damage, protein denaturation, carbohydrate oxidation, pigment breakdown and an impairment of enzymatic activity (Noctor and Foyer, 1998).

The extent of the ROS-induced damage to cells depends on duration and severity of stress. Short-term O_2 deprivation results in a limited accumulation of ROS and lipid peroxidation. In the short-term, the rate of ROS formation and the degree of lipid peroxidation can be regulated by constitutive endogenous antioxidants (Blokhina et al. 2003). In addition, hypoxia induces increased activities of antioxidant systems. Prolonged deprivation of O_2, however, can diminish or even abolish synthesis, transport and turnover of antioxidants. As a consequence of the depleted antioxidants and associated enzymes, cells are unable to cope with the ROS and lipid peroxidation can become severe, particularly during re-oxygenation (see also section 18.6). In addition to causing non-specific increases in membrane permeability resulting from lipid peroxidation, both H_2O_2 and $OH^{\bullet}$ have also been shown to directly control activity of Ca^{2+}- and K^{+}-permeable plasma membrane ion channels (Demidchik et al. 2007, 2010). Perturbations in intracellular ionic homeostasis may initiate programmed cell death (Demidchik et al. 2010).

18.3.3 - Nutrient acquisition by roots in waterlogged soil

Plant nutrient acquisition is dramatically reduced in sensitive species when in waterlogged soil. Upon waterlogging, root growth can be immediately arrested whereas shoots can continue to grow. The resulting increased shoot:root ratio causes an imbalance between shoot nutrient demands and supply by roots. Nutrient ion uptake be roots is also greatly reduced on a per root weight basis (Elzenga and van Veen 2010; Colmer and Greenway, 2011), primarily as a result of reduced O_2 availability inhibiting respiration. Ion uptake by roots consumes energy. The plasma membrane proton pump (H^+-ATPase) requires ATP and the proton motive force generated is used to drive symporter-mediated ion uptake. Indeed, all anions (e.g. NO_3^-) enter root cells via H^+-anion symporters. Furthermore, the H^+-ATPase maintains the negative membrane potential, essential to creating electrochemical gradients allowing channel-mediated uptake of cations (e.g. K^+ uptake). Absence of O_2 inhibited respiration and lowered H^+-ATPase pumping, causing a substantial membrane depolarization, making such cation uptake via channels thermodynamically impossible (Pang and Shabala 2010). Not only is K^+ uptake significantly reduced, but roots can also loose substantial amounts of K^+ through depolarization-activated channels. It is not surprising, therefore, that waterlogged plants often exhibit acute K^+ deficiency. The organic acids present in waterlogged soils, from anaerobic microbial metabolism, can also lead to membrane depolarisation of root cells and reduced ion uptake.

The diminished capacity for ion transport, together with initial ‘dilution’ of shoot nutrient concentrations by continued shoot growth relative to roots, explains a range of nutrient deficiencies observed in leaves of intolerant plants under waterlogged conditions. Waterlogging tolerant species with adequate O_2 supply to roots via large volumes of aerenchyma, can sustain root respiration and therefore plasmamembrane H^+-ATPase functioning for nutrient uptake, as well as having adequate O_2 and energy for deeper root penetration. Efficient internal aeration of roots, together with a barrier to ROL in basal zones, also enables an aerobic rhizosphere at the root tips and regions of dense laterals, altering the rhizosphere (e.g. diminished soil toxins), presumably also with benefits for nutrient uptake by the roots.

18.4 - Internal aeration - aerenchyma and morphological adaptations

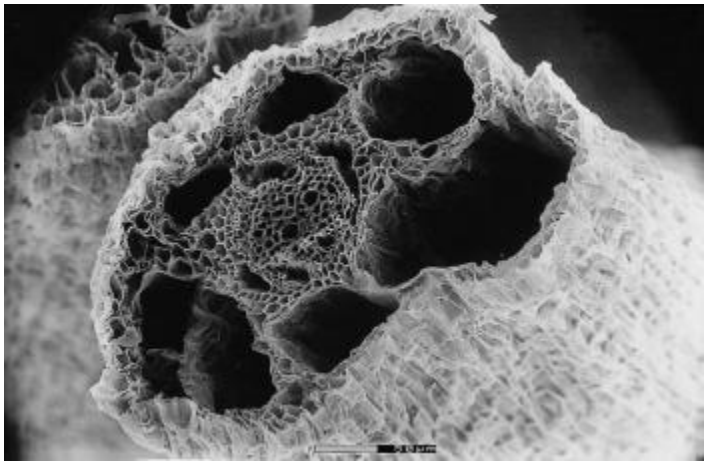


Figure 18.8. Scanning electron micrograph of a root of *Melaleuca halmaturorum* after roots and the lower half of shoots had been flooded for 14 weeks. Extensive aerenchyma have formed through breakdown of cortical cell layers. Scale bar = 100 µm (Micrograph courtesy M. Denton).

Internal O_2 transport from shoots to roots is essential to survival and functioning of roots in anoxic, waterlogged soils. Long-distance O_2 transport through the body of plants occurs via large intercellular gas-filled spaces, termed lacunae or aerenchyma (Figure 18.8).

Movement of gases within root aerenchyma occurs via diffusion, but as will be explained below gas movements can also occur via pressure-driven mass flows in the shoots and rhizomes of some wetland species under certain conditions. When shoots are in air, atmospheric O_2 enters and then diffuses into and along roots. When shoots are completely submerged, tissue O_2 status will change markedly between light (i.e. O_2 produced in photosynthesis during the day) and dark (i.e. night) periods. In summary, aerenchyma provides a rapid gas exchange pathway between the atmosphere and below-ground tissues, essential for survival in flooded environments.

18.4.1 - Aerenchyma in roots

The amount of aerenchyma within roots determines the capacity for internal O_2 transport. Aerenchyma formation is constitutive in roots of many wetland species, although the amount is often further enhanced when soils are waterlogged (Justin and Armstrong, 1987). Many non-wetland species can also form root aerenchyma, but its development can take a couple of days following the onset of waterlogging. Roots of non-wetland species typically, however, form less aerenchyma than wetland species, and some dryland species cannot form aerenchyma. Nevertheless, even in relatively intolerant species such as wheat, newly-formed adventitious roots develop aerenchyma, and these new roots are important since the bulk of the seminal roots die.

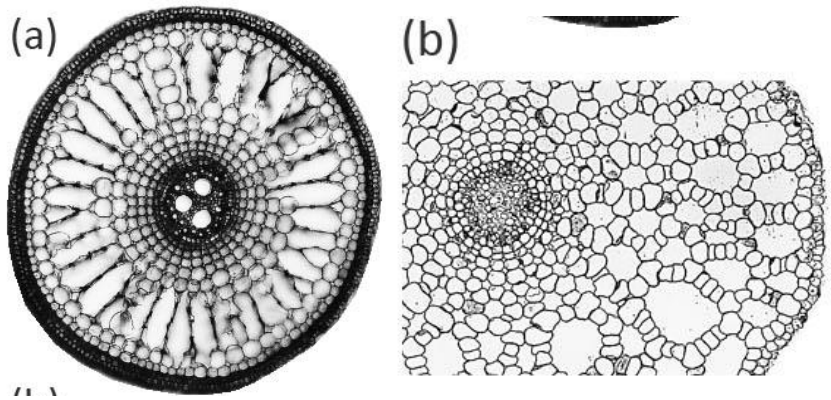


Figure 18.9. Transverse sections of (a) adventitious root of rice showing lysigenous aerenchyma, and (b) lateral root of *Rumex hydrolapathum* showing schizogenous aerenchyma. (a) taken 50 mm behind the root apex, (b) taken 5 mm behind the root apex. (Micrographs courtesy of W. Armstrong).

Aerenchyma can form in the cortex of roots by two main, distinct, developmental processes: (i) lysigeny - the collapse of files of cortical cells to leave behind gas-filled voids (Figure 18.9a), or (ii) schizogeny - cell separation in a radial direction, so that large gas-filled channels form between cells (Figure 18.9b). Lysigenous aerenchyma results from selective programmed cell death in the cortex and this type occurs in many monocots, including important crops (e.g., barley, wheat, maize, rice). Schizogenous aerenchyma is formed by separation of cells (without death) and is found in some wetland species and particularly dicots (e.g., *Rumex* spp.). The 'honeycomb-type' schizogenous aerenchyma shown in Figure 18.9b for *Rumex hydrolapathum* forms due to cells being forced apart owing to oblique divisions by some of the cortical cells in radial rows.

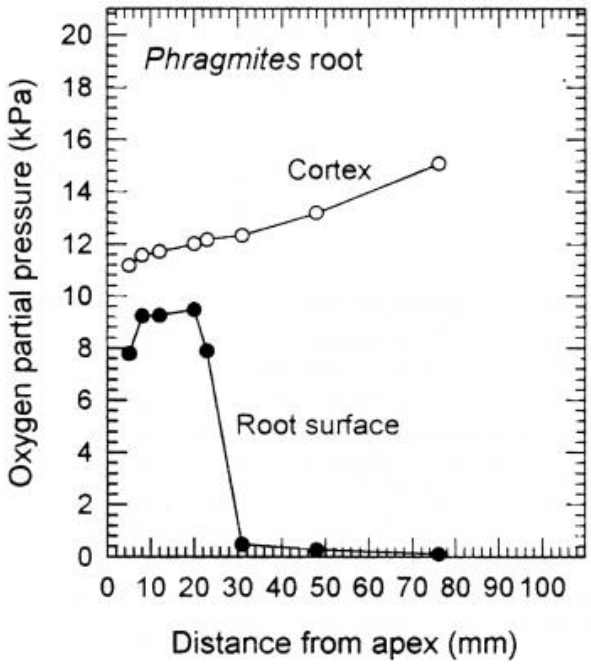


Figure 18.10. Profiles of O_2 with distance behind the apex of the main axis of an intact adventitious root of *Phragmites australis*, when in an O_2-free medium with shoots in air. O_2 within the root cortex increases at positions closer towards the root-rhizome junction, consistent with higher concentrations towards the source of O_2 diffusing down the root. By contrast, O_2 on the exterior of the root surface is highest near the tip, and very low in the basal part of the root. A barrier to radial O_2 loss largely prevents O_2 movement to the rhizosphere in these basal positions (see also description in the text). Jackson and Armstrong (1999).

The involvement of ethylene in formation of lysigenous aerenchyma has been studied in maize roots, and although major gaps exist in understanding of lysigenous aerenchyma, even less is known of the regulation of schizogenous aerenchyma. Evidence for the involvement of ethylene signalling in lysigenous aerenchyma formation is that inhibitors of ethylene action (e.g., silver ions) or of ethylene synthesis (e.g., aminoethoxyvinylglycine, AVG) block aerenchyma formation in hypoxic roots. Hypoxia enhances the activity of an enzyme involved in ethylene biosynthesis (1-aminocyclopropan-1-carboxylic acid (ACC synthase), ACC concentration increased in hypoxic roots, and ethylene synthesis was stimulated. The involvement of ethylene signalling in aerenchyma formation was also supported by experiments in which exogenously supplied ethylene induced aerenchyma formation in aerated roots.

Aerenchyma formation can be quantified by taking root cross-sections and measuring areas of gas-filled spaces relative to the total cross-sectional area. Many studies have quantified root porosity, the gas-filled volume per unit of root volume, and porosity includes the total gas volume in the roots (i.e. large aerenchyma channels plus the smaller intercellular spaces). Porosity in roots of plants grown in waterlogged soil varied from below 1% in some non-wetland species to as much as 53% in one wetland species, demonstrating the wide variation amongst species in their capacities for internal aeration of their roots when in waterlogged soil (Justin and Armstrong, 1987).

The importance of high porosity for root growth in waterlogged soil was demonstrated in a study of 91 species differing in aerenchyma volumes; species with roots of < 5% porosity penetrated only 30-95 mm, whereas those with > 35% porosity grew 150-345 mm into a waterlogged potting mix (Justin and Armstrong, 1987). Mathematical modelling has also highlighted the importance of root porosity for internal O_2 diffusion and therefore root growth in anaerobic substrates (Armstrong, 1979). O_2 supply via aerenchyma determines the respiratory activity and therefore energy status in roots, as demonstrated by measurements of adenylate energy charge (AEC) in root tips of maize seedling roots in an O_2-free medium (Drew et al., 1985). For roots reliant on an internal O_2 supply, the AEC was ~ 0.7 for tips of roots with aerenchyma (porosity ~13%), compared with ~ 0.4 in those without aerenchyma (porosity ~4%). Thus, O_2 supplied via the aerenchyma enables respiration and the ATP produced is essential for the survival, functioning and growth of roots in waterlogged soil.

In addition to large volumes of aerenchyma (i.e. high porosity), roots of many wetland plants also possess a barrier to radial O_2 loss (ROL) in the root exterior (Armstrong, 1979; Colmer, 2003). The ROL barrier diminishes losses of O_2 to the rhizosphere and thus enhances longitudinal diffusion towards the root apex. Loss of O_2 from roots to waterlogged soil can be substantial, owing to the steep concentration gradient from root-to-soil. Many, but not all, wetland species can restrict O_2 losses from the basal parts of their roots. An example of a functional barrier to ROL is shown in Figure 18.10 for a root of common reed (*Phragmites australis*). When in a low O_2 medium, the concentration of O_2 at the root surface was relatively high near the tip, but it was extremely low at 30 mm and further behind the apex. This pattern of low surface O_2 in basal regions, despite the internal O_2 being higher towards

the root base (closer to the O_2 source), indicates a high resistance to ROL across the outer cell layers in sub-apical positions. This resistance to radial O_2 diffusion results from suberin depositions in the hypodermis/exodermis. A 'tight' barrier to ROL develops constitutively in the basal zones of adventitious roots of many wetland species, but was induced by growth in stagnant deoxygenated medium in several others, including rice (Colmer, 2003).

Even for roots with a 'tight' barrier to ROL, losses of O_2 are substantial near the root tip, and also from laterals. ROL around the root tip protects this sensitive growing point from reduced toxins, as these would be re-oxidised as the tip advances into the otherwise anaerobic soil (e.g. Fe^{2+} oxidised to insoluble Fe_2O_3). Thus, ROL may 'protect' the apex against reduced toxins, with the barrier to ROL loss in mature zones not only restricting exit of O_2 but also excluding reduced phytotoxins in the soil.

18.4.2 - Through-flows of O2 along rhizomes of some wetland plants

Pressurised through-flows of gas greatly increase the rate of O_2 transport along rhizomes of several emergent and floating-leaved wetland species, compared to that achieved by diffusion alone. These through-flows increase the concentration of O_2 in rhizomes above those if only diffusion occurred, and the higher rhizome O_2 increases diffusion into roots arising from the rhizomes. Through-flows occur when pressure gradients are established along the aerenchymatous pathway with a low-resistance exit from the plant to the atmosphere (Beckett et al. 1988). Flows can be substantial, for example in the yellow waterlily (*Nuphar luteum*) gas flow rates within aerenchymatous petioles were described by Dacey (1980) as 'internal winds'. Through-flows can result in an increase of two orders of magnitude in the effective length of aeration in culms and rhizomes above that possible via diffusion (Armstrong et al. 1991), enabling some wetland plants to inhabit areas with permanent deep waters and for rhizome growth deep into waterlogged soils. It is important to note that even in species with through-flows along rhizomes, O_2 movement into and along roots occurs via diffusion (the roots are a dead-end side-path so through flows cannot occur without an 'exit').

The importance of through-flows for growing in deep water is especially visible in lakeshore vegetation. On lakeshores, wetland plants such as *Typha* spp. and common reed (*Phragmites australis*) that have flows along rhizomes and grow more deeply than morphologically similar plants that rely solely on diffusive movement of O_2 (Vretare Strand 2002). The deeper the water, the more advantageous it is for a plant to transport gases by pressurised flow rather than diffusion. Emergent plants with efficient through-flows can readily grow in water up to 3 m depth (Sorrell and Hawes 2010), and some floating-leaved plants such as sacred lotus (*Nelumbo nucifera*) are found in up to 5 m water depth.

Rates of through-flow are determined by the pressure gradient and the resistance to flow along the aeration system. The pressure gradient can result from: (i) pressurisation of gas in live shoots due to gradients in water vapour concentration between the interior and exterior of an enclosed space with the surface of the enclosure containing micro-pores (e.g., several wetland species, Brix et al. 1992; Armstrong et al. 1996), or (ii) venturi-induced suction

caused by wind blowing over the open-ends of tall, broken culms so that gas is sucked out, and air enters via shorter culms exposed to lower wind speeds (only documented so far in common reed, *Phragmites australis*, Armstrong et al. 1996). The processes for pressurisation in leaves, and for venturi-induced suction, have been evaluated using physical and mathematical models.

18.4.3 - Specialised roots for flooded environments

Waterlogging tolerant species tend to develop larger adventitious root systems than intolerant species, and these roots contain aerenchyma. The initiation and outgrowth of adventitious roots has been studied in wetland species such as rice and *Rumex palustris*; accumulation of ethylene appears to be the primary signal for this response, and auxin and H_2O_2 are also involved in the downstream signalling cascade (Visser et al. 1996; Steffens and Sauter 2009).

Newly-formed adventitious roots with aerenchyma can grow into anoxic waterlogged soil, but in many cases adventitious roots also grow close to the soil surface, or when floods submerge a significant portion of the stem they emerge into the water column. Surface roots are common for species with low amounts of aerenchyma – the superficial root system is therefore restricted to the surface oxidised layer of the soil. Such plants often develop a 'sprawling' growth form in shallow water with many adventitious roots in the water column, barely entering the soil surface, and taking up nutrients from the surface water. These plants are most common in eutrophic, relatively still water, as they rely on high nutrient concentrations in water given that they cannot exploit soil nutrients. The shallow root system means such plants are vulnerable to uprooting. Aquatic roots that grow into the floodwater are exposed to light and can form chloroplasts, with photosynthesis resulting in high endogenous O_2 levels during the daytime (e.g. Rich et al 2012).

Grey mangroves (*Avicennia* spp.) have long, horizontal roots ('cable roots') close to the soil surface, from which arise hundreds of ca. 1 cm thick, 30 cm long vertical aerial roots termed pneumatophores. O_2 enters the pneumatophores and diffuses via aerenchyma to the underground roots. Even the pneumatophores, however, become temporarily submerged and thus cut off from the atmosphere at high tide. Early researchers suggested that aeration via the pneumatophores might involve pressurised gas flows, but this effect is negligible, and most O_2 transport in mangroves is by diffusion (Beckett et al. 1988). The red mangrove (*Rhizophora* spp.) lacks pneumatophores, instead having aerial 'knee' or 'prop' roots that elevate the trunk above the water surface, and with hypertrophied lenticels serving as entry ports for O_2 which then diffuses to underground roots (Figure 18.11).

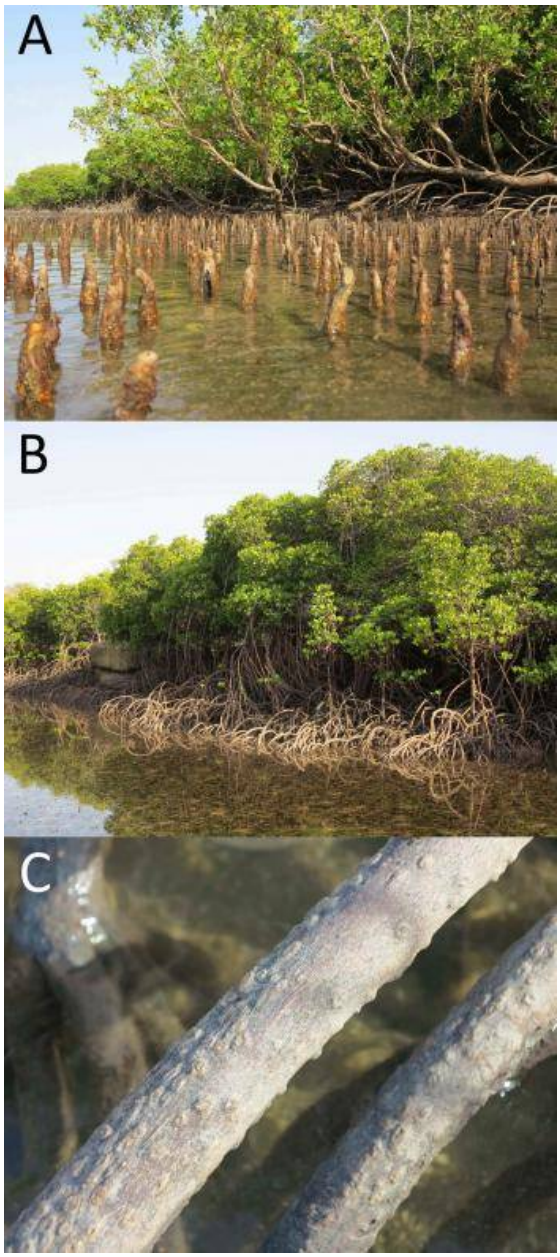


Figure 18.11. Mangroves are trees or large shrubs which grow within the intertidal zone. Mangroves have specialised structures for root aeration, such as (A) pneumatophores (vertical 'air roots') or (B) 'knee roots' (also called 'prop roots'). The surfaces of these roots have lenticels (C; surface of knee roots) which are pores that allow gas exchange between the atmosphere and the internal tissues. At high tide these root structures are submerged whereas at low tide these parts of the roots have direct contact with air and oxygen can then enter via the lenticels. Photographs taken in north-western Australia by Ole Pedersen.

Even with O_2 transport in aerenchyma, trees in wetlands are unable to grow roots as deeply into soil as terrestrial trees, often leaving them vulnerable to toppling. Many flood-tolerant trees in freshwater swamps therefore feature extensions to their lower trunks (buttresses and knees) that provide mechanical stabilisation, and with lenticels present these enable entry of O_2.

18.5 - Complete submergence - escape or quiescence responses

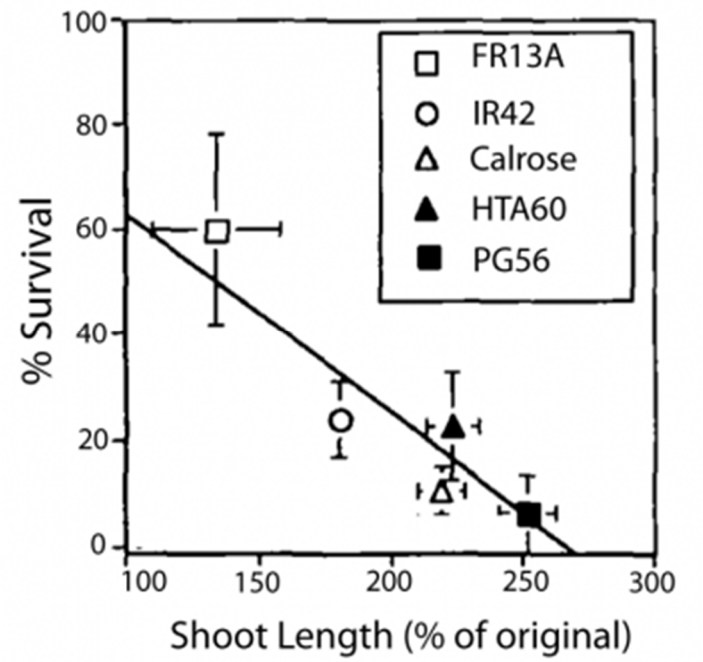


Figure 18.12. Shoot elongation of rice cultivars during submergence relative to survival. Plants were submerged 14 d at a mean daily irradiance of 22.3 ML m-2. Open symbols, lowland rice; closed symbols, deepwater rice cultivars. Setter and Laureles (1996).



Figure 18.13. Petiole elongation response in *Rumex palustris* upon submergence. Plant on the left-hand-side was in air, plant on the right-hand-side was submerged for the final 10 days. (Photograph courtesy of Liesje Mommer).

Leaves, petioles and stems of completely submerged herbaceous plants are generally not anoxic, although can become hypoxic. The internal O_2 concentration in these submerged shoot organs is determined by the rate of photosynthesis under water, the rate of respiration of the tissue and the rate of gas exchange with the external water medium. This results, for example, in endogenous petiole O_2 concentrations in fully submerged *Arabidopsis thaliana* of 17 kPa during the light period and 6 kPa during darkness (Lee et al. 2011; Vashisht et al. 2011). Due to the strong variation over time, O_2 is seen as an unreliable indicator of submergence of shoot organs. Therefore, shoots make use of the entrapment of another gaseous component, the plant hormone ethylene, to sense the change of the outside environment from air to water. Similar to other gases, ethylene only very slowly diffuses in water. Since ethylene is produced by every plant cell, slow diffusion in water leads to a substantial increase of ethylene levels inside shoot tissues within less than one hour of submergence. This enhanced endogenous ethylene concentration is guaranteed in submerged shoots as long as some O_2 is present to maintain the O_2-dependent ethylene biosynthesis.

Flood tolerant plants exposed to complete submergence exploit two contrasting suites of traits, escape or quiescence, to survive this stress. In brief, plants with the escape strategy: (i) increase the growth rate of shoot organs, such as petioles and stems, so as to emerge above floodwaters, and (ii) initiate the development of aerenchyma to facilitate internal gas diffusion. Quiescent plants, on the other hand, “wait out the submergence event” and are characterized by: (i) conservation of energy and carbohydrates via, for example, a reduction of the underwater growth rate, and (ii) an increase of molecular components that prepare shoot and root organs for future conditions with low O_2 and production of protective molecules that counteract harmful cellular changes associated with flooding, such as production of ROS.

A classical study with a range of rice cultivars revealed that shoot elongation under water without reaching the surface goes at the expense of survival and thereby demonstrating elongation is associated with costs (Figure 18.12). From an evolutionary point of view the escape strategy will only persist if these costs are outweighed by benefits such as improved aeration, energy generation and carbon production, and ultimately improved survival, growth and reproduction. Therefore, the escape strategy is restricted to plants in environments with shallow floods or with deeper floods that persist over longer durations as the case with deepwater and floating rice. Transient very deep or ephemeral floods, however, favour plants with the quiescence strategy.

Fast extension of shoot organs in response to submergence is described for species from a wide range of families. As an example, Figure 18.13 illustrates submergence-induced petiole elongation in the semi-aquatic plant *Rumex palustris*.

Depending on the tissue type and the developmental stage of the shoot organ, fast underwater growth involves cell elongation only (e.g. petiole of *Rumex palustris*) or a combination of increased cell division and elongation (e.g. stem of deepwater rice). Fast cell elongation is regulated by specific transcription factors and by an interacting set of plant hormones. In deep water rice, characterized by an enormous stem elongation capacity upon submergence, ethylene regulates two important genes, SNORKEL1 and SNORKEL2, which encode nuclear-localized DNA binding proteins that belong to the Ethylene Response Factor (ERF) family of transcription factors. Non-deepwater rice varieties lack these ERF genes and their importance for elongation was demonstrated by introgression of these loci from deepwater rice into non-elongating varieties which then showed substantial elongation when submerged.

Next to ethylene, three other downstream-operating plant hormones are involved in submergence-induced shoot elongation. Upon submergence, levels of abscisic acid are quickly reduced, whereas auxin and gibberellic acid increase. Ultimately these signal transduction components affect the rate limiting step for cell elongation: the cell wall. In order to allow turgor-driven cell expansion, cell walls must loosen by means of specific cell wall loosening proteins such as Expansins. The expression of Expansin genes is strongly upregulated and the abundance of Expansin proteins increases, soon after submergence of species with shoots that elongate.

An important trait for plants that survive flooding by means of the quiescence strategy is reduction of underwater growth to conserve carbohydrates and retention of chlorophyll to enable continued, albeit reduced, photosynthesis. Increased submergence tolerance in rice caused by reduced plant growth rates under water is regulated by the ethylene-induced expression of the *SUB1A-1* gene. Interestingly, this gene belongs to the same ERF transcription factor family as the two SNORKEL genes. *SUB1A-1* limits elongation growth by two mechanisms: (i) minimizing the decline in the gibberellin signaling repressor SLENDER RICE-1 and the related SLENDER RICE LIKE-1, and (ii) enhancing GA catabolism by differentially regulating genes associated with brassinosteroid synthesis in submerged shoots (Schmitz et al. 2013). On top of that, *SUB1A-1* also inhibits synthesis of ethylene, expression of Expansins and reduces starch and sucrose reserve depletion. Recently, *SUB1A-1* was crossed into high yielding rice varieties leading to more flood tolerant varieties that have recently been released to farmers in Asia. These varieties have yield advantages of 1 to over 3 tons/hectare over the varieties lacking *SUB1A-1* following submergence for various durations (Mackill et al. 2012). Figure 18.14 demonstrates increased survival and yield after 15 d of complete submergence during the vegetative stage followed by recovery after de-submergence of a rice variety containing *SUB1A-1* compared with the original variety that lacks it.

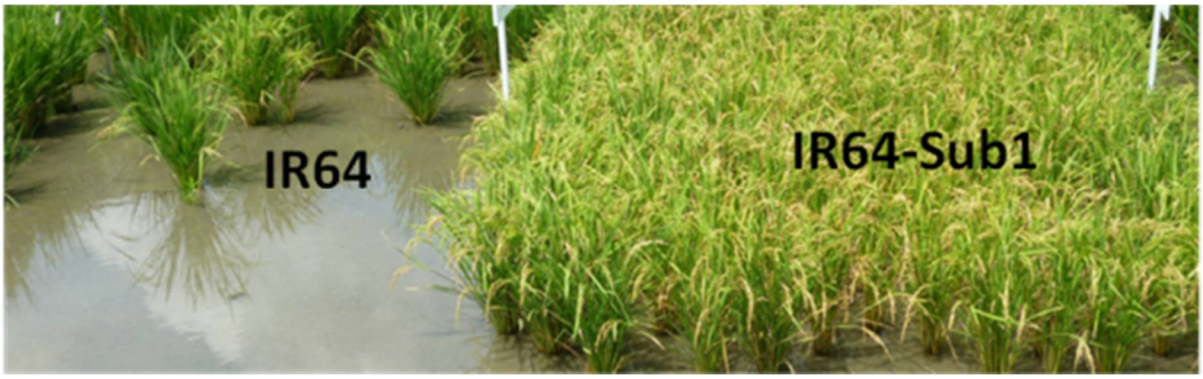


Figure 18.14. IR64 (left) and IR64-Sub1 (right) after 15 days of submergence during vegetative stage in the field. Two-week-old seedlings were transplanted into a field, grown for another two weeks then completely submerged for 15 days. The field was then drained and plants were allowed to recover under non-stress conditions. The photograph was taken about 90 days after de-submergence. (Photograph courtesy of AM Ismail).

In summary, most plants cannot withstand complete submergence lasting over a few days; however, semi-aquatic plants such as certain rice genotypes can survive complete submergence even for over two weeks. Tolerance of rice to transient submergence is mainly achieved by restricting growth and respiration, thus conserving carbohydrate reserves to enhance recovery when the floodwater recedes.

18.5.1 - Photosynthesis under water



Figure 18.15. Leaf gas films on common reed (*Phragmites australis*) during complete submergence. Gas films form on hydrophobic leaf surfaces. Leaf gas films enhance gas-exchange with the surrounding floodwater: CO_2 entry for photosynthesis (in light) and O_2 entry for internal aeration for respiration (in dark), of submerged plants. (Photograph courtesy of Ole Pedersen).

Photosynthesis in completely submerged wetland plants is severely impeded by low light, and the slow diffusion of CO_2 across the aqueous diffusive boundary layer (DBL) adjacent to leaves. Aquatic and amphibious plants have evolved a number of leaf traits to reduce the total resistance to CO_2 uptake, including: (i) dissected leaves, (ii) undulating leaf edges to increase turbulence across the leaf, (iii) thin leaves, (iv) reduced cuticle, and (v) chloroplasts in epidermal cells. The first three traits (i, ii & iii) all serve to erode/reduce the DBL and thus decrease the distance of molecular diffusion (i.e. decreased total external resistance to CO_2 uptake), and (iv & v) reduce the resistance within the tissue for the diffusion pathway to chloroplasts. Likewise, many submerged terrestrial wetland plants also display some acclimation to inundation such as thinner leaves, reduced cuticle and chloroplast orientation towards the source of CO_2. Amphibious plants are positioned between the truly aquatic plants and the terrestrial wetland plants and display a suite of leaf acclimation traits that allows efficient gas exchange by leaves in air, as well as those formed under water.

Rates of net photosynthesis by submerged leaves are typically much lower than rates achieved in air, even for acclimated leaves. Underwater net photosynthesis by submerged terrestrial plants is generally lower than the rates achieved by aquatic plants. However, this is only true when photosynthetic rates are expressed on a per unit area basis, the units commonly used in terrestrial plant physiology. When net photosynthesis is expressed on a per unit dry mass basis, rates in aquatic plants > amphibious plants > terrestrial plants and this order reflects the higher carbon-return per unit of dry mass investment by the aquatic leaf types, as compared with terrestrial leaf types, when submerged.

Gas films on leaves of submerged terrestrial wetland plants have also been shown to facilitate underwater photosynthesis. Gas films form on hydrophobic leaf surfaces of many wetland plants when submerged (Figure 18.15); e.g., species of *Phragmites*, *Typha*, *Spartina*, *Carex*, *Phalaris* and *Oryza* (including cultivated rice), and the gas film forms a large gas-water interface that facilitates gas exchange with the surrounding water.

It is likely that the stomata remain open underneath the gas film. The gas films enable leaves of such terrestrial wetland plants to photosynthesize under water, albeit at rates much reduced when compared with in air, but without further acclimation and this strategy may therefore be particularly advantageous under short floods; as examples, frequent tidal submergence or short duration flash floods such as in some rice-growing areas and natural wetlands where water recedes after a week or two. The improved O_2 and sugar status of submerged rice owing to the beneficial effects of leaf gas films would enhance survival during complete submergence.

Case Study 18.4 - Photosynthesis and internal aeration in submerged aquatic plants

Ole Pedersen, University of Copenhagen, Denmark



Figure 1. Diver operating O_2 and H_2S microelectrodes (left) in a *Thallasia seagrass* meadow in the Caribbean. Development of *in situ* equipment has enabled measurements of internal aeration under challenging field conditions in aquatic systems (right). (Photographs courtesy of Malene Hedegård Petersen (left) and Ole Pedersen (right).

Development of microelectrodes robust enough for use in field conditions has enabled *in situ* measurements of O_2 dynamics in submerged plants (Figure 1). Eco-physiological research on submerged plants has been a challenge; infrared gas analyzers do not work under water! Field studies (*in situ*) have revealed how fluctuating light, diurnal changes in temperature and water column O_2 concentrations all influence internal aeration of submerged aquatic plants. In the

case of seagrasses, application of sulphide microelectrodes has also added to the growing evidence of sulphide poisoning as a likely cause of extensive die-backs.

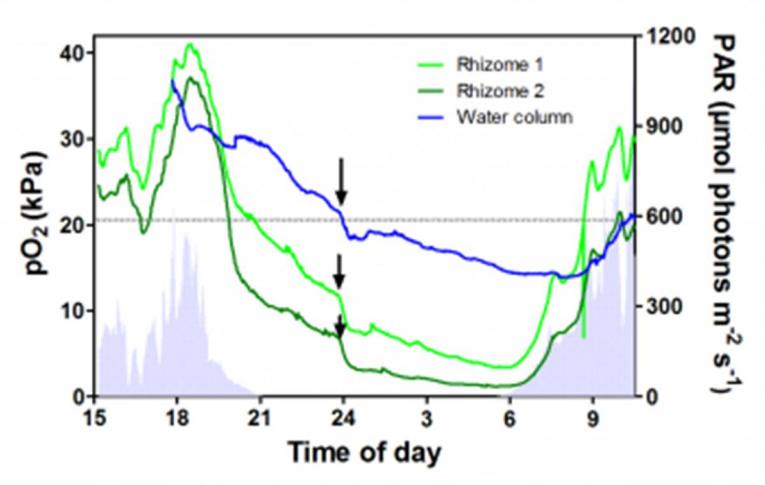


Figure 2. Oxygen dynamics in the rhizome of the seagrass *Zostera marina* during a diurnal cycle. O_2 in the rhizome closely follows incoming light during daytime, whereas during the night internal aeration relies on supply of O_2 from the water column. The dependence on water column pO_2 during the night for internal aeration becomes particularly clear when the tide carries water across the seagrass meadow with lower pO_2 as the internal pO_2 of the rhizomes immediately declines (see arrows). During the day, it is the other way around as fluctuations in incoming light is immediately reflected in rhizome pO_2 as daytime rhizome pO_2 follows underwater photosynthesis. Data from Greve et al. (2003).

Seagrasses are flowering plants with roots, rhizomes, sometimes stems, and almost always strap shaped leaves to reduce pressure drag and thus the uprooting forces created by wave action. Seagrasses are key marine ecosystem engineers and habitat for various marine animals, but seagrasses are under world-wide threat from human activities (eutrophication, dredging and other physical disturbances). Eutrophication impacts directly on seagrasses by decreasing the available light (stimulates growth of epiphytes and planktonic algae) but also indirectly by stimulating the decomposition of organic matter in the sediment (mineralization of algae and seagrass litter is often limited by N and P) and thus increases the O_2 demand of the sediment. In marine sediments, sulphate reduction by microorganisms can be substantial under anoxic conditions, producing sulphide, a potent phytotoxin with toxicity and mode of action similar to that of cyanide. Sulphide exists in three different chemical forms in water (H_2S, HS^- and S^{2-}) with the gaseous H_2S dominating at low pH and S^{2-} at high pH. Gaseous H_2S can enter the root and rhizome aerenchyma and move via diffusion to other parts of the seagrass, such as to leaf meristems that are relatively sensitive to sulphide. The resulting sulphide poisoning is a major cause of the worldwide die back of seagrasses observed in temperate as well as in tropical seagrass meadows.

Mechanistic field studies employing *in situ* microelectrodes have improved our understanding of internal aeration and sulphide intrusion in natural seagrass meadows. During the day, internal aeration of roots and rhizomes relies on O_2 production in underwater photosynthesis, as does radial O_2 loss (ROL) from roots to the sediments. There is a strong relationship between incoming light and O_2 partial pressure in roots (Figure 2). Clouds immediately lead to a decline in root O_2, whereas during periods of high sunlight root O_2 was highest. During the night time, however, internal aeration relies on a steady flux of O_2 from the water and into the leaves, and via the aerenchyma, further down into the roots and rhizomes.

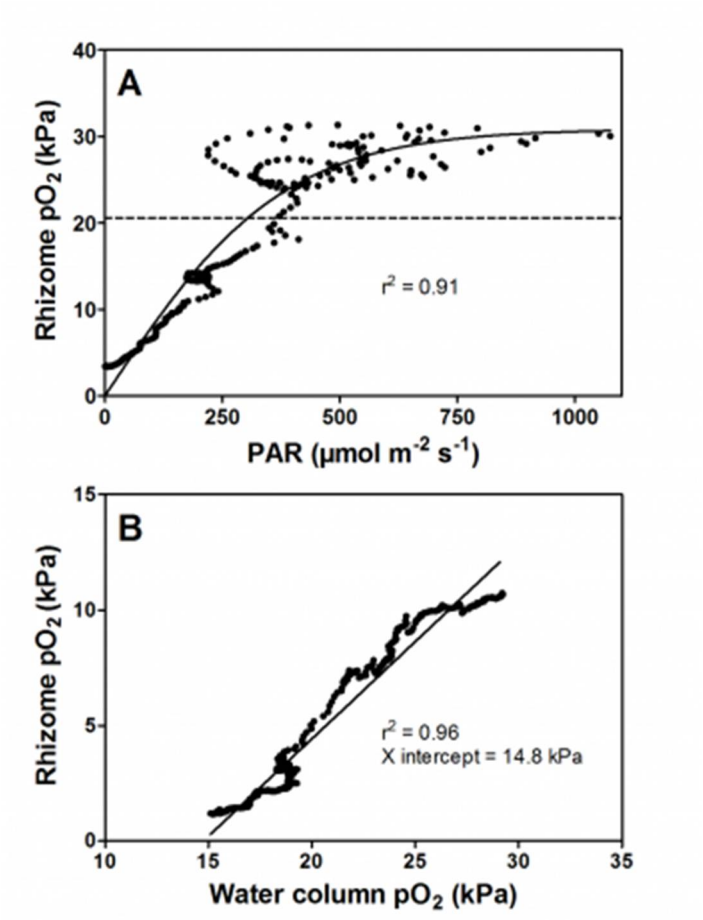


Figure 3. Oxygen dynamics in the rhizome of the seagrass *Zostera marina* during the day (A) and during the night (B). During the day, underwater photosynthesis produces O_2 in the leaves and rhizome pO_2 is thus a function of incoming light as O_2 easily diffuses from the chloroplast and into the root via the porous tissues. In contrast, during the night, the water column is the only source of O_2 for the plant and O_2 diffuses from the water and into the leaves and further down into the belowground tissues. Consequently, rhizome pO_2 is strongly correlated to water column pO_2 during darkness. Re-analyzed data from Greve et al. (2003).

Critically low water column O_2 can occur during nights in areas with still, warm waters, resulting from net system respiration faster than inwards movement of O_2 into the stagnant waters. Under such night time conditions, roots can experience anoxia. Cessation of ROL to the sediments means there is no longer chemical oxidation of H_2S to SO_4^{2-} in the rhizosphere, so that gaseous H_2S enters the aerenchyma and spreads via gas phase diffusion to all parts of the seagrass. The very metabolically active leaf meristems are thought to be particularly sensitive to sulphide poisoning. It is thought provoking that during most sudden die backs of seagrasses, the shoots are found drifting in the water with apparently healthy leaves but detached from the vertical stem exactly where the basal leaf meristems is located.



Figure 4. *Lobelia dortmanna* and other isoetids in a Norwegian oligotrophic lobelia lake. The water of such lakes contains only little dissolved CO_2 but *L. dortmanna* can take up CO_2 from the sediment where the concentration is typically 100-fold higher. As a consequence, *L. dortmanna* has no barrier to radial O_2 loss (ROL) in its roots and almost all O_2 produced in underwater photosynthesis is lost to the sediment via the roots. (Photograph courtesy of Ole Pedersen).

In contrast to coastal marine habitats of seagrasses, lobelia lakes are highly transparent oligotrophic, low alkaline lakes of the northern hemisphere. The vegetation consist of several evergreen species that are morphologically strikingly similar: short stiff leaves arranged in a rosette and with unbranched roots that can make up more than 50% of the biomass. As a whole, the type of vegetation is referred to as isoetids from the genus *Isoetes* that occurs in most lobelia lakes. Although not present in all lobelia lakes, *Lobelia dortmanna* is key species (Figure 4). Isoetids take up CO_2 from the sediment via the roots and some are even CAM plants, although conservation of water is probably the least of all concerns for these plants. CO_2 concentrations are highest at night, so CAM enables storage of malate for subsequent decarboxylation providing CO_2 for photosynthesis the next day.

Many sandy sediments in shallow lobelia lakes are permanently oxic. Oxic sediments are a consequence of inherently low mineralization rates as the oligotrophic conditions lead to very low production of organic matter that is subsequently decomposed in the sediment. Nevertheless, the CO_2 concentration in these sediments can be 100-fold higher than in the water above and *L. dortmanna*, along with the other isoetids, tap into this rich source of CO_2 with their large root systems. The large gradient in partial pressure of CO_2 between sendiment and photosynthetic leaves drives a flux of CO_2 from the sediment, into the root aerenchyma (radial CO_2 uptake) and then upwards into the porous leaves. Interestingly, the leaves are covered with a relatively thick cuticle to prevent loss of CO_2 to the surrounding water, and as a result up to 100% of the O_2 produced in underwater photosynthesis is lost via ROL from the roots (Figure 5).

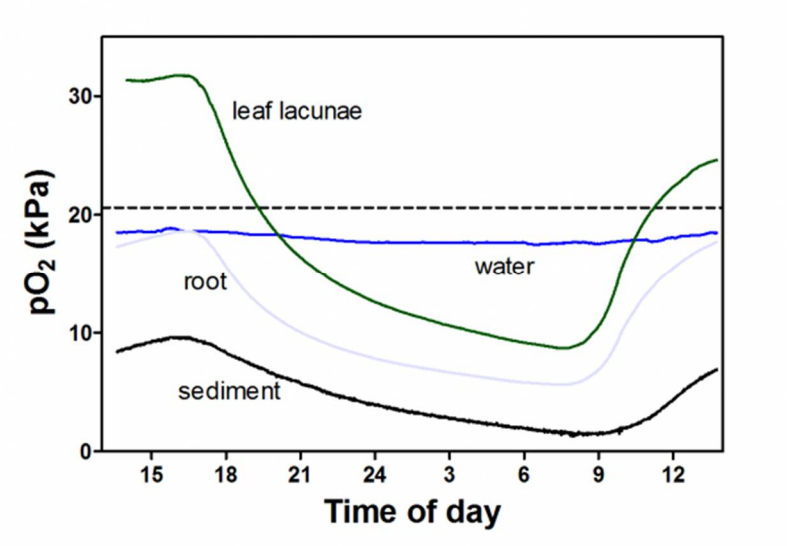


Figure 5. Oxygen dynamics in leaves and roots of *Lobelia dortmanna* and the surrounding water column and sediment in Lake Värsjö, Sweden. In light, underwater photosynthesis drives leaf pO_2 up above 30 kPa and the steep gradient to roots results in a substantial O_2 flux into roots. The roots have no barrier to ROL and thus the majority of O_2 produced in photosynthesis is lost to the sediment resulting in large diurnal fluctuation in sediment pO_2; in fact, the sediment remains permanently oxic. Data modified from Sand-Jensen et al. 2005.

In conclusion, the isoetids tested so far do not form a barrier to ROL in their roots and isoetids are thus restricted to sediments with very low O_2 demand; any root barrier would also restrict CO_2 uptake. In contrast, the few species of seagrasses studied all show a strong barrier to ROL and in the marine H_2S rich environment the barrier would also reduce the inward flux of gaseous H_2S.

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18.5.2 - Internal aeration when completely submerged

During daytime, underwater photosynthesis not only provides sugars but it also produces O_2 which results in significant aeration of belowground tissues. In light, the partial pressure gradient of O_2 from shoot to root is huge (high O_2 in the surrounding water, zero O_2 in the anoxic soil) and can thus drive a substantial flux of O_2 from shoot to root in well-developed aerenchyma as this pathway poses relatively little resistance to diffusion. In fact, O_2 in the roots of rice displays a normal saturation curve relationship when plotted against light available to the shoots, a pattern also found in truly aquatic plants (see Case Study 4).

At night, the floodwater surrounding the shoot is the main source of O_2 for internal aeration also of belowground tissues. The O_2 "stored" in aerenchymous tissues cannot support night time respiration of belowground tissues as the O_2 in the aerenchyma relatively quickly equilibrates with the environment (soil in the case of roots and water column in the case of shoot). However, with ample O_2 in the floodwater a large partial pressure gradient exists for O_2 movement from the floodwater into the leaves and further into the roots (both respiring and thus consuming O_2). As a result, the relationship between floodwater O_2 and root pO_2 in darkness is often linear (see Case Study 4). The floodwater O_2 threshold concentration required for O_2 to enter and reach the root extremities is determined by the total resistance to molecular diffusion into (DBL, surface gas films, stomatal resistance, cuticular resistance) and through the plant body (tissue porosity, diffusion distance) plus loss of O_2 along the route (respiration and any ROL). As with underwater photosynthesis, leaf gas films reduce the resistance to gas exchange between floodwater and leaves, so enhancing O_2 entry at night-time. Plants with gas films have been shown to maintain better internal aeration as compared to plants with the gas films experimentally removed.

18.6 - Recovery when waters recede

Most terrestrial plants cannot withstand complete submergence lasting over a few days; however, semi-aquatic plants such as certain rice genotypes can survive complete submergence for over two weeks, as described in the preceding section. The period of submergence endured depends on various environmental conditions and the plant's growth stage.

Tolerance in rice of transient submergence caused by flash floods is mainly achieved by assuming a "quiescent" strategy when submerged until floodwater recedes. Recovery after submergence when floodwaters recede is dependent on metabolic changes that occur during and immediately following submergence.

18.6.1- Shoot desubmergence

As detailed in Section 18.5, complete submergence restricts light intensity and gas exchange, slows O_2 and CO_2 exchange between shoot tissue and floodwater. Reduced photosynthetic activity, together with excessive growth during submergence, result in severe carbohydrate starvation and consequently, death and disintegration of most tissues when flooding persists for longer duration.

Visual symptoms of stress generally start developing soon after desubmergence, with sensitive genotypes showing leaf senescence and decay, followed by mortality within a few days after desubmergence.

Excessive growth during submergence is common, and due to accumulation of the phytohormone ethylene. Submerged plants tend to elongate excessively, an "elongation escape" adaption that allows their leaves to maintain contact with air until the floodwaters are too deep. This elongation capacity is mediated through ethylene which suppresses ABA synthesis but enhances synthesis and sensitivity to GA, resulting in leaf and internode elongation (Das et al. 2005). Ethylene accumulation also triggers chlorophyll degradation and leaf senescence (Ella et al. 2003b), rendering leaves less fit for photosynthesis both underwater and upon resumption of contact with air after desubmergence.

The sudden aeration and exposure to high illumination upon desubmergence causes oxidative stress resulting from ROS generated in leaves that have limited capacity for photosynthesis following submergence (Ella et al. 2003a).

Recovery after submergence therefore, depends on maintenance of carbohydrate reserves, during and shortly after flooding (Das et al., 2005), and the maintenance of a functional photosynthetic system. In rice, as explained in Section 18.5, tolerance of submergence is conferred by an ethylene response-like transcription factor *SUB1A*. Induced by ethylene that accumulates within plants during submergence, *SUB1A* disrupts the elongation escape strategy typical of most lowland rice varieties through suppressing GA-promoted elongation, and also slows ethylene-induced leaf senescence (Bailey-Serres et al. 2008). Survival and

recovery are enhanced in two ways: (i) less energy is consumed on elongation growth and carbohydrates are conserved, and (ii) leaf senescence is prevented. Thus, plants continue photosynthesis while underwater, and can resume optimal rates of carbon fixation upon re-exposure to air and high illumination and so minimise ROS damage after desubmergence. The sudden exposure to high O_2 and high light increases the generation of ROS. The ability to recover quickly and produce new tillers following desubmergence is important because only these new tillers will become effective in contributing to grain yield.

18.6.2 - Soil drainage following waterlogging

Re-oxygenation injury is well-documented for both animal and plant tissues. A highly reduced intracellular environment (including transition metal ions) and low energy supply, such as occurs during soil waterlogging, are the factors which favour ROS generation. Free radicals are formed soon after O_2 re-enters, in a so-called oxidative burst. At the same time, activity of most plant antioxidant systems is compromised due to metabolic perturbations caused by the previous period of anoxia or severe hypoxia (Blokhina et al. 2003). Production of ROS upon reaeration might impede ion uptake by roots; H_2O_2 causes membrane depolarization and K^+ efflux (Chen et al. 2007). Recovery of nutrient uptake upon re-aeration following anoxia in roots of wheat showed a short time lag (~ 4 h) before net K^+ uptake accelerated. The time lags could have been associated with repair of general metabolism (e.g. lag in recovery of mitochondria), repair of membranes and membrane transporters, or the prevention of damage from ROS.

Recovery of root growth upon drainage is important for crops in rain-fed agriculture, as deep roots will be required to obtain sufficient water from the soil later in the season (e.g. wheat in Mediterranean climates). Seminal roots of wheat cease growing soon after waterlogging, and if waterlogging exceeds several days these roots show little capacity for re-growth upon drainage and soil aeration. Apices of the main axes of the seminal roots typically die, with any regrowth from laterals. Adventitious roots, by contrast, are able to grow in waterlogged soils and retain the potential of the main axes to elongate upon soil aeration. Resumption of growth of adventitious roots following drainage can be fast, so that these roots continue in their importance for future shoot growth. Nevertheless, the adventitious root system could not compensate for the severe inhibition of the seminal roots of wheat. For waterlogging-sensitive crops, even short periods of transient waterlogging can have longer-term adverse effects. In wheat, for example, 3 d waterlogging severely retarded development even in the longer term after drainage (Malik et al. 2002), highlighting the need for improved waterlogging tolerance in our crops.

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