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A method to estimate practical radial oxygen loss of wetland plant roots

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Abstract

The estimation of practical radial oxygen loss (ROL) of wetland plant roots was attempted in this study. We have devised a new method to measure ROL of wetland plant roots. The whole root system was bathed in an anoxic nutrient solution. Oxygen released from the root was removed immediately by introducing oxygen-free nitrogen gas (O_2 < 4 nmol L^{-1}) to mimic natural habitats where released oxygen is consumed rapidly due to chemical and biological oxidation processes. Oxygen removed from the root-bathing chamber was simultaneously detected colorimetrically by use of the highly oxygen-sensitive anthraquinone radical anion ($AQ^{\cdot -}$) in a cell outside the root-bathing chamber, which decolorized by a rapid reaction with oxygen. An emergent macrophyte *Typha latifolia* L. was incubated, and its ROL was measured by both the new method and one of the conventional methods, the closed chamber/electrode method, by which the ROL of *Typha latifolia* L. had not yet been measured. The new method succeeded in detecting the ROL, whereas the conventional method was not able to detect oxygen, due to the level being below the detection limit of the oxygen electrode. The oxygen supply via the seedlings of *Typha latifolia* L. was ca. 10 times higher compared with control measurements without plant. Light illumination significantly enhanced the ROL of *Typha latifolia* L. (0.33 nmol O_2 g^{-1} root dry weight s^{-1} under light and 0.18 nmol O_2 g^{-1} root dry weight s^{-1} in the dark). Theses values fall between those previously reported by the closed chamber/titanium citrate method and the open chamber/electrode method.

Abbreviations: Anthraquinone – AQ; Anthraquinone radical anion – $AQ^{\cdot -}$; ROL – radial oxygen loss

Introduction

Radial oxygen loss (ROL) from the roots tips of wetland plants has been demonstrated in numerous studies. This accounts for the strategic mechanism of wetland plants against hypoxic habitats. In order to tolerate anoxic problems, many wetland plants develop aerenchymatous lacunar that serve as a pathway of oxygen transport from aboveground to underground parts (Armstrong, 1972; Justin and Armstrong, 1987; Teal and Kanwisher, 1966). Oxygen in leaves diffuses toward roots with low resistance through continuous lacunae. Furthermore, some species develop another more effective mechanism (through-flow convection) driven by a pressure differential to transport oxygen to their underground parts (Armstrong and Armstrong, 1990; Brix et al., 1992; Dacey, 1980, 1981; Grosse, 1996). Oxygen molecules transported to roots are not only used for respiration, but they also diffuse to the rhizosphere and form an oxidative layer around the root surfaces. This oxidative layer prevents plant absorption of phytotoxic reduced substrates such as Fe^{2+}, Mn^{2+} and sulfide (Armstrong and Armstrong 1988; Christensen et al., 1994; Conlin and Crowder, 1988; St-Cyr and Crowder, 1989).

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Differently from monotonically anoxic unvegetated sediment, vegetated sediment thus forms an oxidative-anoxic mosaic (Armstrong et al., 1992). Accordingly, the activated microorganisms metabolic processes around the interface of the oxidative rhizosphere and the anoxic non-rhizosphere affect elemental cycles such as those of carbon and nitrogen throughout the entire wetland ecosystem (Arth et al., 1998; Arth and Frenzel, 2000; Bodelier et al., 1996; Bosse and Frenzel, 1997; Frans-Jaco et al., 1998; Frenzel et al., 1992; Gilbert and Frenzel, 1998; Lombardi et al., 1997; Reddy et al., 1989a, b). The exact evaluation of ROL is therefore required to assess the role and the impact of aquatic plants on wetland ecosystems.

Several methods have been employed to evaluate ROL from roots. *In situ* evaluation of ROL is quite difficult, because numerous heterogeneous factors are involved in the oxygen-consuming processes. Analyzing oxidized Fe^{3+} plaques on root surfaces (Mendelssohon and Postek, 1982) and measuring increased redox potential *in situ* in vegetated sediments (Carpenter et al., 1983; Chen and Barko, 1988; Jaynes and Carpenter, 1986; McKee et al., 1988) have provided valuable information of the ROL impact on natural soils. However, the amount of oxidized Fe^{3+} plaques and the increase in redox potential substantially fluctuate according to the types and availability of chemical compounds as well as the amount of ROL. Several quantitative methods in a laboratory study have also been proposed, but each method has some specific problems. Monitoring net oxygen efflux from whole root systems bathed in deoxygenated solutions in a closed chamber with an oxygen electrode sensor (Sorrell and Dromgoole, 1987), which we designate here as the closed chamber/electrode method, may give underestimated results, ascribed to a less steep oxygen decrease gradient around the root and re-absorption by other parts of the root (Sorrell and Armstrong, 1994). In natural sediments, oxygen is consumed by chemical and biological oxidation processes immediately after being released from roots (DeLanune et al., 1990; Lorenzen et al., 1998; Teal and Kanwisher, 1966). Bedfold et al. (1991) proposed a method using an open system with an oxygen electrode sensor (open chamber/electrode method) to measure the net effect of the plant as both source and sink for oxygen. The oxygen-depleted solution is continuously flowed through the root-bathing chamber, and the difference in the oxygen concentration between the inflow and outflow solution is monitored. However, the flow rate of the oxygen-depleted solution flowing through the root-bathing chamber is extremely low due to the detection limitation of the oxygen electrode sensor. This causes high oxygen concentration in the root-bathing chamber and hence underestimation has also been a concern (Sorrell and Armstrong, 1994). Kludze et al. (1994) and Sorrell and Armstrong (1994) proposed another method, which does not make use of an oxygen electrode sensor (closed chamber/titanium citrate method), and which prevents accumulation of oxygen around the root and well mimics the soil redox potentials in nature. These authors used Ti^{3+} citrate solution as an oxygen scavenger. The blue-violet color of Ti^{3+} citrate, which is a strong reducing agent, is decolorized in the process of consuming oxygen. The solution of Ti^{3+} citrate is added to the closed root-bathing chamber, and the amount of Ti^{3+}-consumed oxygen in the chamber is evaluated colorimetrically. However, in a long-term measurement, the high salinity of the titanium citrate solution (ca. 84‰ NaCl) may impose a confounding treatment effect on salt-sensitive plant species (Lissner et al., 2003). In order to overcome these problems, another procedure free from oxygen accumulation around the root and free from any phytotoxic substrate is needed.

We have devised a new method to measure ROL resolving these problems using the highly oxygen-sensitive anthraquinone radical anion ($AQ^{\cdot -}$) as an oxygen detector (open chamber/anthraquinone radical anion method). The purpose of this study is to confirm whether the new method is adequate to measure ROL by comparing with the values obtained from the previous methods for an emergent macrophyte, *Typha latifolia* L. This plant exists ubiquitously in wetlands, and its ROL has already been evaluated by the open chamber/electrode method (Bedfold et al., 1991) and the closed chamber/titanium citrate method (Jespersen et al., 1998) but not yet by the closed chamber/electrode method. We evaluated its ROL by the closed chamber/electrode method and the open chamber/anthraquinone radical anion method in this study.

Materials and methods

Plant material

Seeds of *Typha latifolia* L. were collected from fallow fields in Chiba City, Japan, in November 2002 and germinated in a container filled with sediment which was collected from Lake Inbanuma, 20 km north of Chiba City in March 2003. The plants were transplanted to grow in the experimental field at Chiba University in 2004. When shoots reached 100 mm in height, each shoot was separated in each growth pot system. The growth pot system consisted of two parts (Figure 1). The upper part was a PVC tube (170 mm tall, 60 mm in diameter) covered with a nylon net (5-mm mesh) lid on the bottom. The lower part was a 1 L glass pot. The PVC tube was mounted on the center hole of the plastic lid of the glass pot. The plant was established in the PVC tube containing 420 mL sediments (150 mm in depth), and the water depth was maintained at 10 mm throughout the growth period. The glass pot was filled with sediment suspension (sediment:water = 2:3) and covered with aluminum foil to prevent light penetration. After 2 weeks, the plants had developed healthy root systems penetrating through the nylon net into the underlying glass pots. After removing the glass pot, the exposed root system was rinsed gently to serve the ROL measurements. All of the experimental plants were 461 ± 39 mm in shoot height and had 5.5 ± 1.2 leaves (mean $\pm$ SD, $N = 4$).

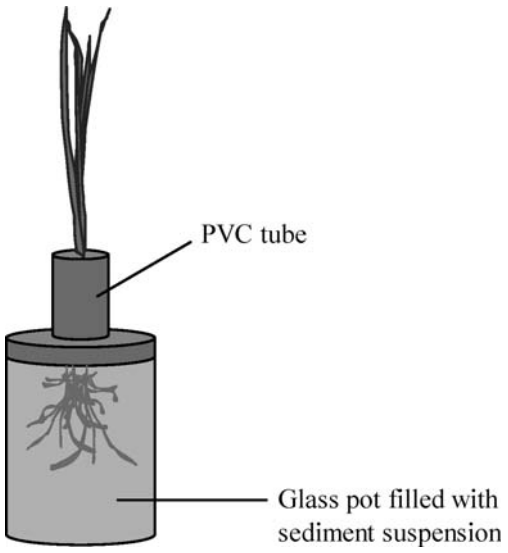

Figure 1. Schematic diagram of the growth pot system. The upper part of the PVC tube can be easily detached from the lower part, i.e., the glass pot.

Measurement of ROL

Four replicate plants in PVC tubes were assigned to the following ROL measurements. First, the plant was assigned to the new method (open chamber/anthraquinone radical anion method). The ROL under dark conditions after 10 h pretreatment and then that under illuminated conditions (850 μmol photon m^{-2} s^{-1} measured at the shoot base) after 2 h pretreatment were measured. The amounts of oxygen released from the root system within 300, 600, 900 and 1200 s were determined. The ROL (mol O_2 g^{-1} root dry weight s^{-1}) was obtained as a regression coefficient of the amount of the oxygen released from the root versus time.

After the measurements by the open chamber/anthraquinone radical anion method, the plant was assigned to the closed chamber/electrode method. The plant was stripped from the PVC tube, and its root system was rinsed gently with tap water in order to remove any sediment which might act as a sink for oxygen. The ROL measurements were conducted in the dark after 10 h pretreatment and then under illuminated conditions after 2 h pretreatment. The amount of the root newly exposed to the nutrient solution after rinsing was less than 1% of the total root mass. We therefore ignored the difference in exposed root amount between the two methods. After all of the measurements were completed, the root system was oven-dried at 80 °C for 72 h and weighed to evaluate the ROL on a root dry weight base.

Open chamber/anthraquinone radical anion method

In this method, the red colored anthraquinone radical anion ($AQ^{\cdot -}$)/isopropyl alcohol solution was used as an oxygen detector. Anthraquinone solution has been known to serve as an oxygen indicator, and the technique has been referred to as photonometric titration (Kuwana, 1963). The UV light-excited anthraquinone (AQ*) abstracts a β-hydrogen from isopropyl alcohol to form the semiquinone radical (Eq. 3), which undergoes a

deprotonation to afford the red-colored anthraquinone radical anion ($AQ\cdot^-$) in alkaline solution (Eq. 5).

$$AQ + hv \rightarrow AQ^*(S) \quad (1)$$

$$AQ^*(S) \rightarrow AQ^*(T) \quad (2)$$

$$AQ^*(T) + (CH_3)_2CHOH \rightarrow AQH\cdot + (CH_3)_2\dot{C}OH \quad (3)$$

$$(CH_3)_2\dot{C}OH + AQ \rightarrow AQ\dot{H} + (CH_3)_2C = 0 \quad (4)$$

$$AQH\cdot + OH^- \rightleftharpoons AQ\cdot^- + H^+ \quad (5)$$

where AQ*(S) and AQ*(T) are anthraquinone in the excited singlet state and triplet state, respectively and AQH· is the anthrasemiquinone radical. In the presence of oxygen, the red-colored $AQ\cdot^-$ is oxidized to colorless AQ with an almost diffusion-controlled rate constant (ca. $k = 10^{10}\ s^{-1}\ M^{-1}$).

$$AQ\cdot^- + O_2 \rightarrow AQ + O_2^- \quad (6)$$

A series of these reactions form the cycle represented in Figure 2. The solution therefore enables us to use the ROL measurement repeatedly,

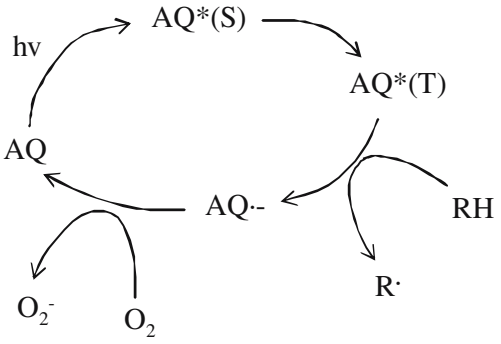

Figure 2. Reaction scheme of anthraquinone solution. AQ: anthraquinone; AQ*(S): anthraquinone in the excited singlet state; AQ*(T): anthraquinone in the excited triplet state; $AQ\cdot^-$: anthraquinone radical anion; RH: isopropyl alcohol.

based on the restoration of the oxidized AQ to $AQ\cdot^-$ by UV irradiation.

The measurement apparatus consisted of three components: (a) a nitrogen gas regulator (AM-080, STEC, Japan; capacity 0–33.3 mL air s^{-1}), (b) a root-bathing chamber and (c) an oxygen detection section (Figure 3). The entire gas-introduction system consisted of glass and stainless steel tubing. The PVC tube with an experimental plant was inserted through the center hole of the lid of the root-bathing chamber, which contained a 700-mL nutrient solution. The whole root system was submerged in the nutrient solution, which was continuously sparged with oxygen-free nitrogen gas ($O_2 < 4$ nmol L^{-1}) through a glass filter (100–120 μm mesh). The flow rate of nitrogen gas was regulated at 5 mL s^{-1}. The solution was vigorously stirred with a Teflon-coated magnetic-stir bar at the bottom of the chamber, and a magnetic stirrer was equipped outside the chamber. The root-bathing chamber was maintained at 20 °C throughout the measurement. The oxygen concentration of the nutrient solution was monitored throughout the measurement by a Clark-type oxygen electrode sensor (UD-101E, Central Science, Tokyo, Japan) furnished beside the root system to ensure that the oxygen released from the root was thus immediately removed. The removed oxygen was transported by the nitrogen gas flow to 25 mL of the red colored anthraquinone radical anion ($AQ\cdot^-$)/isopropyl alcohol solution in the cell equipped outside the root-bathing chamber. The volume of the cell was 50 mL; a schematic diagram of the cell is represented in Figure 4. Since the red color of the $AQ\cdot^-$ solution is decolorized by the rapid reaction with oxygen, the peak absorbance at 512 nm of the $AQ\cdot^-$ solution was determined by an UV/visible spectrophotometer (U1000, Hitachi, Tokyo, Japan) to obtain the amount of $AQ\cdot^-$ consumed. During the introduction of the sample gas to the $AQ\cdot^-$ solution, the gas-introduction tube was inserted down to the cell bottom. Every 5 min, the gas-introduction tube was removed from the cell, and the cell was immediately mounted in the spectrophotometer to measure absorbance. Each trial was conducted in 20 s. Since the isopropyl alcohol solvent evaporated slightly as the nitrogen gas was introduced (70 nL s^{-1}), the volume of the $AQ\cdot^-$ solution in the cell at the time of the absorbance measurement used for the calculation was

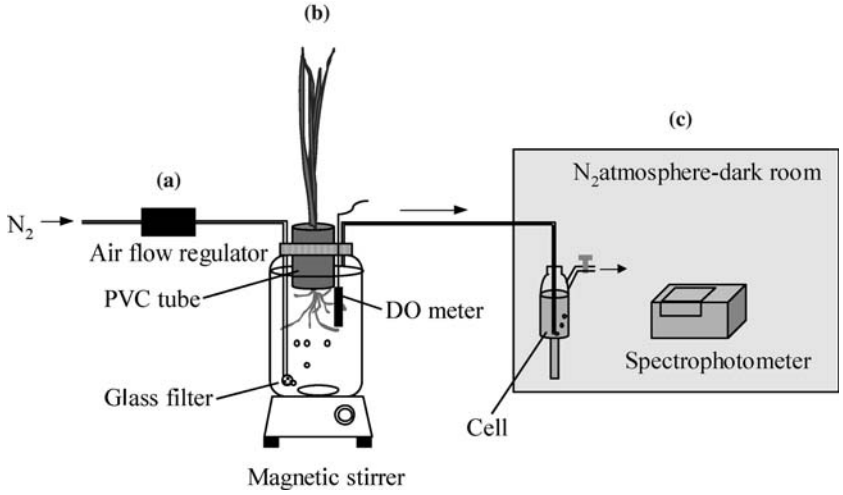

Figure 3. Schematic diagram of the experimental apparatus used to measure the oxygen released from the root system by the open chamber/anthraquinone radical anion method. The main components of the system are: (a) a flow regulator for oxygen-free nitrogen gas; (b) a root-bathing chamber; and (c) a detection section. The entire gas-introduction system consists of glass and stainless steel tubing. The arrows in the figure represent the direction of the gas flow. See Materials and methods for details.

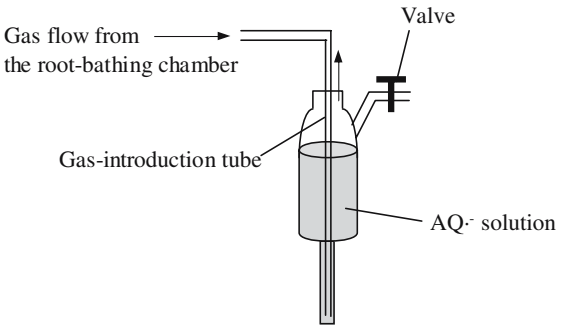

Figure 4. Schematic diagram of the cell used for the measurements. The arrows in the figure represent the direction of the gas flow. The volume of the cell is 50 mL. To introduce the gas from the root-bathing chamber to the $AQ^{\cdot -}$ solution, the gas-introduction tube (6mm in diameter) was carefully inserted down to the cell bottom. The gas-introduction tube was raised above the $AQ^{\cdot -}$ solution during the measurement of the absorbance.

corrected. The cell was kept in the dark and in the oxygen-free N_2 atmosphere throughout the measurement.

The practical extinction coefficient (ε_0) of $AQ^{\cdot -}$ at 512 nm in this experimental setup was determined to be 2.00×10^6 L mol^{-1} m^{-1} by the following procedure. Replacing the PVC tube, a rubber stopper was placed on the root-bathing chamber of the measurement apparatus, and nitrogen gas was introduced in the same way as in the sample measurement. Aliquots of oxygen-saturated water of 0, 0.2, 0.4 and 0.6 mL were injected into the root-bathing chamber by a syringe through the rubber stopper, and the resultant decrease in absorbance of the $AQ^{\cdot -}$ solution was measured. Since $AQ^{\cdot -}$ reacts with an equimolar amount of oxygen at a nearly diffusion-controlled rate (Bolland and Cooper, 1953; Tickle and Wilkinson, 1965; Wilkinson, 1962), the amount of the injected oxygen X [mol O_2] was calculated according to the following equation:

$$X = \Delta\mathrm{ABS} \cdot \frac{V}{\varepsilon_0 l} \tag{7}$$

where ΔABS is the difference of the absorbance at 512 nm before and after the nitrogen gas sparging, respectively, and V is the volume of the $AQ^{\cdot -}$/isopropyl alcohol solution (around 25 mL) and l is the path length (10 mm). Therefore, ε_0 is obtained as the slope of the regression of ΔABS vs. X (Figure 5) multiplied by V over l.

The measurements were conducted so that the absorbance of the $AQ^{\cdot -}$ solution was above 0.5. Since the oxygen molecule is consumed according to the reaction in Eq. 6, the lifetime of oxygen τ [s] is estimated by the following equation:

$$\tau = \frac{1}{k[\mathrm{AQ}^{\cdot -}]} \tag{8}$$

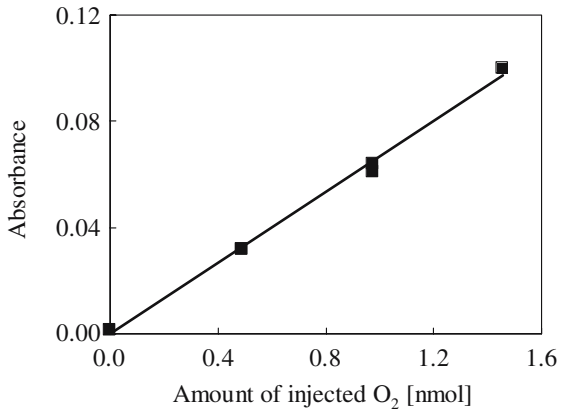

Figure 5. Correlation between the amount of oxygen injected into the apparatus and the decrease in absorbance of the $AQ^{\cdot -}$ solution at 512 nm. The slope of the linear regression of the decrease in absorbance vs. the amount of injected O_2 was used for the calculation to obtain the extinction coefficient (ε_0).

where k is the rate constant of the reaction in Eq. 6. Supposing $k = 10^{10}\ s^{-1}\ M^{-1}$ and $[AQ^{\cdot -}] = 25\ \mu M$ (initial absorbance at 512 nm, 0.5; $\varepsilon_0 = 2.00 \times 10^6\ L\ mol^{-1}\ m^{-1}$), τ is calculated to be 4 μs, which should be negligibly short compared to the residence time for the oxygen molecule in the nitrogen flux after being sparged with nitrogen into the bottom of the cell.

The $AQ^{\cdot -}$/isopropyl alcohol solution was prepared under an N_2 atmosphere by the following procedure. A 50% isopropyl alcohol (250 mL) solution was added to 250 mL of a 0.2 mM anthraquinone 2–6 disodium sulphonate (TCI, Tokyo, Japan) solution, and the pH was adjusted to 11 with a 1 M sodium hydroxide solution. The solution was irradiated with a UV lamp (FL 15BL-B, National, Japan, 365 nm) to convert the anthraquinone (AQ) to the red-colored $AQ^{\cdot -}$.

Closed chamber/oxygen electrode method

A whole root system was submerged in the oxygen-depleted 25% strength Hoaglands' nutrient solution (700 mL, 20 °C). The oxygen electrode sensor (UD-101E, Central Science, Tokyo, Japan) was used to monitor the oxygen accumulation in the solution. The rate of oxygen exchange was calculated from the change in the amount of oxygen in the chamber over time (Sorrell and Dromgoole, 1987).

Statistical analysis

The data obtained were subjected to a *t*-test to determine the significance of differences between treatments, after the homogeneity of the variance was tested.

Results

The ROL values evaluated for *T. latifolia* were quite different among the four methods proposed (Table 1). The closed chamber/electrode method did not detect the ROL of *T. latifolia*, as the oxygen concentration in the root-bathing chamber remained below the detection limit for the oxygen electrode of 0.3 μmol $O_2\ L^{-1}$ for more than 3 h. This corresponds to below 0.01 nmol $O_2\ g^{-1}$ root dry weight s^{-1}.

The open chamber/anthraquinone radical anion method detected a decrease in absorbance of the $AQ^{\cdot -}$, although the oxygen electrode detected no increase in oxygen concentration around the root system throughout the measurement. Figure 6 shows a representative absorbance chart for the measurement. A decrease in the absorbance indicates the oxidation of the red-colored $AQ^{\cdot -}$ to colorless AQ by the oxygen introduced from the root-bathing chamber. Although the available $AQ^{\cdot -}$ decreased as the measurements were repeated, oxidized AQ was restored to $AQ^{\cdot -}$ by UV irradiation (indicated by an arrow in the chart) to continue the measurements.

Under the control treatment (furnished with sediment in the chamber containing a PVC

Table 1. ROL of *T. latifolia* obtained by several methods

Method	Oxygen release (nmol $O_2\ g^{-1}$ root dry weight s^{-1})	Reference
Closed system		
Oxygen electrode	<0.01[a]	This study
Titanium(III) citrate	33.3–55.6	Jespersen et al. (1998)
Open system		
Oxygen electrode	0.01–0.16	Bedfold et al. (1991)
Anthraquinone radical anion	0.18–0.33	This study

[a]ND, not detected.

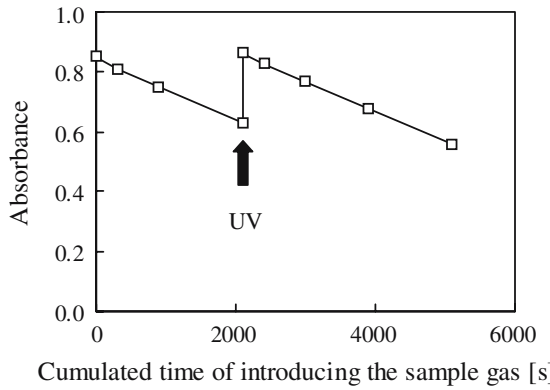

Figure 6. Chart trace of absorbance of the $AQ^{\cdot -}$ solution at 512 nm vs. time, in the presence of a root system in the root-bathing chamber. Symbols represent trials for the absorbance measurements. A decrease in absorbance indicates the oxidation of red-colored $AQ^{\cdot -}$ to colorless AQ by the oxygen introduced from the root-bathing chamber. The arrow indicates UV irradiation to reconvert AQ to $AQ^{\cdot -}$.

tube with no plant), oxygen diffusion from the atmosphere to the root-bathing chamber was 0.01 nmol O_2 m^{-2} s^{-1} (SD = 0.00, $N = 5$), less than one tenth of the oxygen supply via the seedlings of *T. latifolia* ($P < 0.01$). The ROL of *T. latifolia* under the illuminated condition was 0.33 nmol O_2 g^{-1} root dry weight s^{-1} (SD = 0.09, $N = 4$), which was nearly twice as high as that (0.18 nmol O_2 g^{-1} root dry weight s^{-1}, SD = 0.05, $N = 4$) under the dark condition (Figure 7, $P < 0.05$).

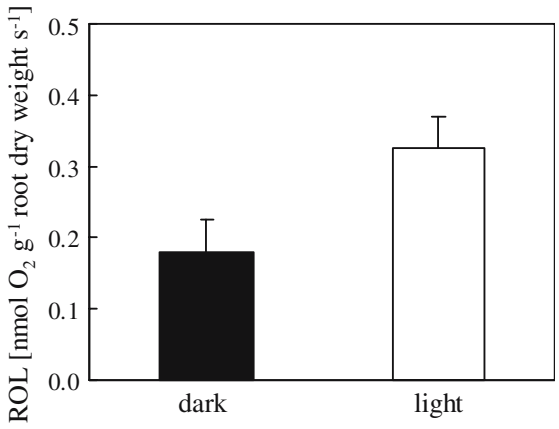

Figure 7. ROL of *T. latifolia* in the light (850 μmol photon m^{-2} s^{-1} measured at the shoot base) and dark (0 μmol photon m^{-2} s^{-1}). Values are the mean ± 1 SE ($N = 4$).

Theses values fall between those previously reported by the closed chamber/titanium citrate method and the open chamber/electrode method. The value obtained from the open chamber/electrode method was smaller than that of the open chamber/anthraquinone radical anion method. For the closed chamber/titanium citrate method, the value obtained was ca. 100 times higher than that of the open chamber/anthraquinone radical anion method.

Discussion

ROL measurement

In order to estimate a practical ROL in a laboratory study, the oxygen conditions around the root should be set up to be similar to naturally occurring conditions. The concentration gradient between the root surface and the sediment is the critical factor determining the ROL. *In situ* measurements proved that the oxygen concentration beside an oxygen-releasing root surface ranged from 40 to 150 μmol O_2 L^{-1} and dropped below the detection limit of the oxygen electrode sensor at distances of a few mm from the oxygen-releasing root (Arth and Frenzel, 2000; Gilbert and Frenzel, 1998). For the closed chamber/electrode method, oxygen released from the root was not removed throughout the measurement. This must have resulted in a less steep gradient of oxygen profile around the root and may have led to underestimated results. Furthermore, it has been a concern that released oxygen might have a possibility to diffuse into a neighboring root where the oxygen concentration is significantly lower than the ambient solution and be consumed in respiration (Sorrell and Armstrong, 1994). ROL thus was sometimes not detected, as shown in this study. In the open chamber/electrode method, ROL was detected, but the value obtained was lower than those of two of the other methods, the closed/titanium citrate method and the open chamber/anthraquinone radical anion method. The oxygen concentration in the root-bathing chamber (ranging from 10 to 30 μmol O_2 L^{-1}) was extremely high (Bedfold et al., 1991) compared with that in natural sediment. Hence, as in the case of the closed chamber/electrode method, the oxygen decrease gradient around the

root must be less steep than that under natural conditions, and underestimation had already been a concern (Sorrell and Armstrong, 1994).

For the closed chamber/titanium citrate method, the value obtained was excessively high. Since Ti^{3+} is a strong reducing agent, adding it directly into the root-bathing chamber might result in steeper gradient of oxygen around the root than with the other methods and thus the high ROL value was obtained. Lissner et al. (2003) suggested that titanium-treated plants must be stressed with the high salinity (ca. 84‰ NaCl) of this solution. The salinity effect might be negligible for a short-term ROL measurement, but in a long-term measurement, the salinity could inhibit water uptake and respiration, which results in wilting and decreasing oxygen transport to underground parts and thus decreased ROL. In addition, the plant might absorb the strong reducing agent Ti^{3+} from the nutrient solution. The absorbed Ti^{3+} can consume oxygen even in the roots and leaves of the plant.

In the new method proposed here (open chamber/anthraquinone radical anion method), the oxygen concentration in the root-bathing chamber was controlled to maintain it below the detection limit of the oxygen electrode sensor, as in natural conditions, throughout the measurement. We prevented oxygen accumulation around the root, which can be the case in the open chamber/electrode method, based on the use of the highly oxygen-sensitive anthraquinone radical anion ($AQ^{\cdot -}$) as a detector. This enabled us to sparge nitrogen gas vigorously through the root-bathing chamber and to achieve a sufficiently low oxygen concentration around the roots. Furthermore, a very small amount of ROL can be detected by extending the measurement time, since this method detects the accumulation of ROL during the measurement time period.

ROL impact of T. latifolia

Frenzel et al. (1992) demonstrated that 80–90 % of the methane produced in sediment was oxidized by methane-oxidizing bacteria in the rice rhizosphere. Furthermore, a positive correlation between the presence of oxygen-releasing roots and the activity of the nitrifying bacteria in the root zone had been demonstrated (Bodelier et al., 1996; Reddy et al., 1989a). More evidence for nitrification–denitrification between the interface of the oxidative rhizosphere and the anoxic non-rhizosphere was reported in several investigations (Arth et al., 1998; Reddy et al., 1989b). Similar processes must occur in the rhizosphere of dense *Typha* stands. In addition, oxidized substrates resulting from ROL also affect microorganism activities in the rhizosphere. A high concentration of Fe^{3+}-containing oxide in the rhizosphere has been evaluated in several investigations (Green and Etherington, 1977; Mendelssohn and Postek, 1982; St-Cyr and Crowder, 1989; Roden and Wetzel, 1996). Fe^{3+}-reducing bacteria use this Fe^{3+} as an electron acceptor to oxidize organic compounds. Roden and Wetzel (1996) reported that microbial Fe^{3+}-oxide reduction suppressed sulfate reduction and methanogensis. Van Bodegom et al. (2001) designed a mechanistic model describing the kinetics of aerobic oxidation of the most important electron donors and the diffusion of these donors from a rice root surface. This model predicted that 80% of the ROL in the rice rhizosphere would be consumed by the chemical oxidation of Fe^{2+}, while 15% would be consumed by aerobic heterotrophs. According to this, Fe^{3+} produced by *T. latifolia* is estimated to be 1.1 nmol Fe^{3+} g^{-1} root dry weight s^{-1} in the light (850 μmol photon m^{-2} s^{-1}) and 0.6 nmol Fe^{3+} g^{-1} root dry weight s^{-1} in the dark.

In order to evaluate practical ROL impact on wetland sediments, we should obtain more information about ROL dependence on numerous factors. It has been reported that illumination enhances ROL in various aquatic plants (Caffrey and Kemp, 1991; Carpenter et al., 1983; Connell et al., 1999; Frenzel et al., 1992; Kemp and Murray, 1986; Lorenzen et al., 1998; Pedersen and Sand-Jensen, 1995; Sand-Jensen et al., 1982), including *T. latifolia* in this study. High concentrations of photosynthetically derived oxygen are thought to enhance oxygen transport and thus ROL (Caffrey and Kemp, 1991; Connell et al., 1999; Frenzel et al., 1992). Light can also decrease pressurization and convective gas flow rate, probably as a consequence of the enlarged stomatal opening, as for *T. angustifolia* (Bendix et al., 1994). This lowered pressurization can decrease oxygen transport by through-flow convection to the roots in *T. angustifolia*, although this is still unknown. Indeed, in *T. latifolia*, light

did not decrease pressurization of leaves (Bendix et al., 1994). In *Phragmites australis* (Cav.) Trin ex. Steud., opening of stomata might enhance the humidity-induced diffusion, because when the stomata are closed, projecting wax obscures the openings and decreases the humidity gradient across the pores (Armstrong and Armstrong, 1990, 1991). In *T. latifolia*, light might therefore have a positive effect on ROL by the raised oxygen concentration in leaves and convective gas through-flow.

Acknowledgements

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