THE EFFECT OF KEY PHYSIOLOGICAL FEATURES ON ROOTS OXYGEN RELEASE OF FIVE WETLAND VEGETATIONS

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Abstract. Macrophytes are considered essential components of constructed wetlands. Previous research has indicated that oxygen supplied by wetland plants is important for wastewater treatment. However, the capacity of wetland plants to release oxygen, and its importance, is debatable. This paper compares the capacity of five wetland plants to release oxygen from their roots, and explores the physiological and morphological reasons for this ability. The results reveal significant differences between five wetland plant species in the same environment. *Phragmites australis* released more oxygen (maximum release rate was from 253.0 to 424.2 μmolg^{-1} h^{-1}) into the substrate than did *Typha orientalis* Presl. did (maximum release rate was from 74.7 to 273.8 μmolg^{-1} h^{-1}). Root oxygen release rate correlated positively correlated with physiological and morphological features including root porosity (POR), shoot length, chlorophyll-a, and dry root biomass. The eco-physiological features and morphologies of Gramineae species were beneficial for releasing oxygen from roots. Our results may be useful in guiding those responsible for selecting plants to be included in constructed wetlands.

Keywords: *macrophytes, morphological, root porosity, Gramineae species*

Introduction

Constructed wetlands (CWs) have long been recognized as effective, low-cost, and eco-friendly instruments to remove a wide range of wastewater pollutants (Zhang et al., 2022; Kirui, 2016). Aquatic plants play an important role in wetland succession and are considered to be essential components of CWs (Brix, 1994). Firstly, nutrients and other contaminants (Teuchies et al., 2012), such as micro-pollutants and heavy metals, can be taken up by macrophytes for reproduction and plant growth (Gacia et al., 2019; Wang et al., 2021). Secondly, the existence of plants can help to improve the hydraulic conductivity in CWs (Brix, 1994). Thirdly, they may facilitate bacterial attachment and growth by increasing the available surface area of roots, and with it enhance the diversity and abundance of microorganisms in the system (Kulshreshtha et al., 2022). Finally, the root systems of macrophytes exude a range of degradable organic compounds, such as amino acids, organic acids, and sugars. Carbon can be provided continually to denitrification bacteria in the modeled constructed wetland.

Oxygen released from plant roots plays an important role in the contaminant removal from CWs (Kadlec et al., 2009). The importance and capacity of oxygen supplied by plants in CW systems is presently in debate. Capacity varies in accordance with growth conditions and the plant species itself. Growth conditions include humidity, air and water temperature, light intensity, air pressure and so on (Armstrong and Beckett, 1990;

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Stottmeister et al., 2003; Bezbaruah and Zhang, 2004a; Soda et al., 2007; Zhang et al., 2014). Sorrell's research results suggest that the external oxygen demand control radial oxygen loss (ROL) at low to moderate reducing intensities (Sorrell, 1999). It has been shown that ROL is positively correlated with rates of transpiration and photosynthetic (Lai et al., 2012). Bezbaruah and Zhang (2004a) demonstrated that the amount of root oxygen released varies with the level of oxygen stress present in the rhizosphere. Another disadvantageous factor is the plant's defense mechanism against phytotoxins (Bezbaruah and Zhang, 2004b). In conditions of static water levels, higher oxygen release rate because of the continuous flooding. The significant decrease in root porosity and ROL is due to changes in water level (Sasikala et al., 2009). Oxygen release rate from roots is related to morphological and physiological characteristics of wetland plants (Cheng et al., 2014).

Lai et al. studied the relationships between pollutants removal, photosynthesis, and ROL, with lots of species. The results revealed that positively, the transport effectiveness of gas in the aerenchyma of plant, diffusion correlated with POR, and POR significantly affected root oxygen release (Lai et al., 2012). Wieβner et al. (2002) revealed that oxygen release intensities were found to vary between the species. Their research indicated that root oxygen release rate was also affected by total biomass, leaf and root biomass, maximum length of root, photosynthetic and transpiration rate, and activity of root. All indicators were significantly correlated with plant growth.

The differences among the plant species were significant, but the capacity of root oxygen release from different species, and the reasons for this, remain unclear (Vymazal et al., 2021). The aim of the present study was to compare the root oxygen release ability of five wetland plants: *Acorus calamus* Linn., *Typha orientalis* Presl., *Thalia dealbata*, *Phragmites australis*, and *Pontederia cordata* Linn., and explore the morphological and physiological reasons for the phenomena. Our results may guide the selection of plants for CWs, and further knowledge into the mechanisms of nutrient removal in these sites.

Materials and methods

Plant material

Five different species: *Acorus calamus* Linn., *Typha orientalis* Presl., *Thalia dealbata*, *Phragmites australis*, and *Pontederia cordata* Linn. were used in this study. The macrophytes were collected from the shores of Mochou Lake, Nanjing, China (32°4′17″N, 118°47′29″E). All aquatic plants were taken manually without damage (Keddy, 2010). The species were selected based on the catalogue of Angiospermae (*Table 1*).

Table 1. *The wetland plant species*

Science name	Family	Genus
Acorus calamus Linn.	*Araceae*	*Acorus*
Phragmites australis	*Gramineae*	*Phragmites*
Pontederia cordata Linn.	*Pontederiaceae*	*Pontederia*
Typha orientalis Presl.	*Typhaceae*	*Typha*
Thalia dealbata	*Marantaceae*	*Thalia*

Experimental reactor and procedures

Oxygen release from plants was determined with phytotoxic-free titanium (Ш) citrate buffer (Dong et al., 2011); oxygen release tests were performed in deoxygenated reducing solutions (Sorrell and Armstrong, 1994; Jespersen et al., 1998). Titanium (Ш) citrate (0.2249 g citric acid, 8 ml $TiCl_3$) was added to distilled water and purged with N_2 gas to remove dissolved oxygen.

The roots of the five different species were then submerged in the citrate buffer in five different jars. In order to prevent re-aeration from the atmosphere, poured a 15-mm layer of paraffin oil on top of the solution, ensuring that the only possible source of oxygen within the chamber was roots of plant. The basal part of all shoots and chambers were wrapped with tinfoil. Jars were kept outside of the laboratory. Luminometer (MODEL ZDS-10F-2D) was used to measure the light intensity every 1 h (*Fig. 1*). Average value of light intensity, temperature and humidity during experiments were 498 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 22 °C and 28%, respectively.

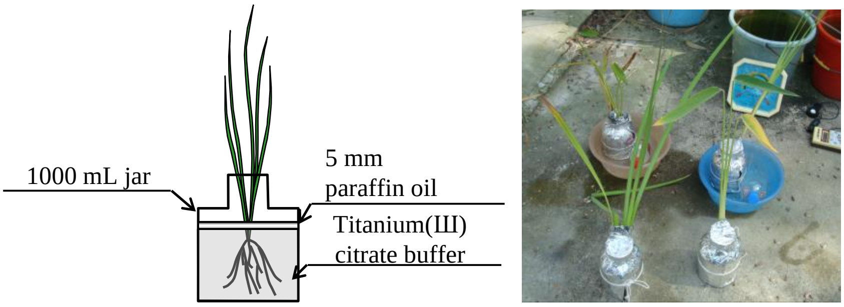

Figure 1. *Device for detection of root oxygen release*

Measurement of oxygen release.

According to the reduction rate of Ti^{3+}, the oxygen release from the root can be obtained. Ti^{3+} is oxidized by oxygen released from roots. Samples were taken every 1 h and measured with a spectrometer at a wavelength of 527 nm. Absorbencies were compared with standard curve (Dong et al., 2011). *Equation 1* shows the reaction between O_2 and Ti^{3+} (Kang et al., 2006). It reveals that 4 moles of Ti^{3+} are consumed when 1 mole of O_2 is reduced. The consumed oxygen (ΔO_2, mg) can be calculated by *Equation 2:*

$$O_2 + 4Ti^{3+} + 4H^+ = 4Ti^{4+} + 2H_2O \tag{Eq.1}$$

$$\Delta O_2 = \frac{32 \times V \times (C_0 - C_e)}{4 \times 47.73} \tag{Eq.2}$$

where C_0 are initial Ti^{3+} concentrations, C_e are end Ti^{3+} concentrations, and V is the volume of titanium (Ш) citrate buffer solution, 0.9 L. The rate of root oxygen release (V_O, $\mu mol g^{-1} h^{-1}$) can be calculated by *Equation 3:*

$$V_O = \frac{\Delta O_2 \times 1000}{24 \times 32 \times Dry\ root\ weights} \tag{Eq.3}$$

Measurement of physical signs

POR (Porosity, %, gas volume/root volume) was measured using the pycnometer method and 25 ml pycnometers (Jensen et al., 1969; Mei et al., 2014). All debris was removed from the roots by vacuum cleaning before measurement. Fresh roots of the five species ranged from 0.41 g to 2.0 g. Porosity was calculated using the *Equation 4:*

$$porosity(\%) = \frac{W_h - W_{r+w}}{W_w + W_r - W_{r+w}} \times 100 \qquad \text{(Eq.4)}$$

where W_h = mass of pycnometer with water and vacuumed roots in *g*; W_{r+w} = mass of pycnometer with water and fresh roots in *g*; W_w = mass of water-filled pycnometer in *g*; W_r = mass of fresh roots in *g*.

Chlorophyll-a content of leaves was determined spectrophotometrically (UV 1900, Shimadzu) in 80% acetone (Arnon, 1949; Gao et al., 2014). Dry weights of plant roots (or shoot or leaf) were examined after drying for 24 h at 80 °C. The surface area of leaf was examined with graph paper. The porosity of a shoot or leaf was measured using the method given in the previous paragraph.

Statistical analysis

(1) Diurnal fluctuation parameters in root oxygen release

Root oxygen release can be modelled using Gaussian function (Dong et al., 2011). This represents a unimodal distribution. *Equation 5* can describe diurnal fluctuation in root oxygen release basing on the Gaussian function.

$$V_O = ae^{-\frac{(t-t_{Omax})^2}{c^2}} \qquad \text{(Eq.5)}$$

Here *a* represents the maximum oxygen release rate for one day and one night, and *c* is the oxygen release rate of the Gaussian function gradient, and *t* is time, 4:00–20:00.

Light intensity data also follow a Gaussian function:

$$PAR = be^{-\frac{(t-t_{Lmax})^2}{d^2}} \qquad \text{(Eq.6)}$$

Formula b is the PAR peak per day, d is the gradient of the Gaussian function, PAR is photosynthetically active radiation, $\mu mol \cdot m^{-2} \cdot s^{-1}$.

(2) Correlation coefficients

Correlation analysis, mean and standard deviation were performed using SPSS.

Results

Figure 2 shows the daily release of oxygen from roots of five different wetland plant species under the same light intensity on 2, 3 and 6 October. PAR was photosynthetically active radiation. In the morning, the oxygen release from roots increased with the increase of light intensity. At noon, the amount of oxygen released decreases with the decrease in light intensity. At night, the oxygen release rate of the roots is almost zero.

The peak value of maximum oxygen release rate for *Acorus calamus* Linn., *Typha orientalis* Presl., *Thalia dealbata*, *Phragmites australis*, and *Pontederia cordata* Linn. were in the range of 150.65-609.63, 74.72-273.84, 256.53-325.65, 253.03-424.17, and 154.65-285.65 μmol g^{-1} h^{-1}. And the average value was 328.44, 193.03, 291.19, 364.38, 212.32 μmol g^{-1} h^{-1}, respectively. The ability of the roots of *Phragmites australis* and *Acorus calamus* Linn. to release oxygen was higher than that of the other species. The data showed a significant difference in oxygen release rate between the five wetland plant species.

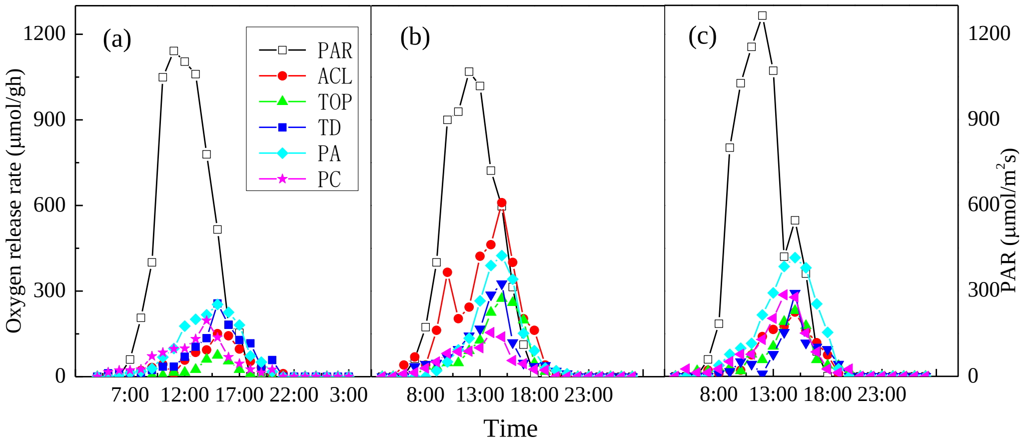

Figure 2. *Different oxygen release rates of five wetland plant species under the same light intensity: (a) 2 October; (b) 3 October; (c) 6 October*

Quantitative analysis of the diurnal variation of the oxygen release rate within the roots was performed. The parameters of the Gaussian function are shown in *Table 2*. The *c* value of steep Gaussian function is small, while the *c* value of gradually varying Gaussian function is large. A larger *c* parameter means that more data values were higher, in accordance with the peak value. However, if the parameter *a* was low, the oxygen release rate would be low. It cannot be influenced by parameter *c*. The results (*Table 2*) revealed that maximum rate of oxygen release of *Phragmites australis* was higher than that from *Typha orientalis* Presl. All data and parameters were comprehensively analyzed, and the capacity of root oxygen release for the five plant species were: *Phragmites australis* > *Acorus calamus* Linn. > *Thalia dealbata* > *Pontederia cordata* Linn. > *Typha orientalis* Presl.

Table 2. *Modeling parameters**

Plant species Parameters	***Acorus calamus* Linn.**	***Typha orientalis* Presl.**	***Thalia dealbata***	***Phragmites australis***	***Pontederia cordata* Linn.**
a	274.1 ± 147.8	187.8 ± 86.3	232.6 ± 33.5	359.7 ± 79.2	201.2 ± 20.0
c	3.5 ± 0.36	2.4 ± 0.24	2.7 ± 0.39	3.2 ± 0.46	4.0 ± 0.06

*The 24 h light intensity was fitted by Gaussian function, where *b* and *d* are calculated to be 1187 ± 68.9 and 3.2 ± 0.09 μmol·m^{-2}·s^{-1}, respectively

Table 3 displays the eco-physiological and morphological features of five aquatic plant species, and the correlation coefficients between physical signs and oxygen rate. Based on the Correlation coefficients values, root oxygen release rate was significant positively correlated with some main factors, such as porosity, chlorophyll-a, dry root biomass, dry leaf biomass, shoot length, and root diameter. The oxygen release rate was negatively correlated with leaf porosity, root length, and root number. There were no significant correlations between oxygen release rate and shoot porosity, leaf surface area, or dry shoot biomass.

***Table 3.** Physical signs of 5 wetland plant species*[*]

Plant species **Physical signs**	***Acorus calamus* Linn.**	***Phragmites australis***	***Thalia dealbata***	***Typha orientalis* Presl.**	***Pontederia cordata* Linn.**	**Correlation coefficients**
Shoot porosity (%)	6.8	47.8	49.7	17.8	73.2	-0.1670
Leaf porosity (%)	49.5	17.0	29.5	71.8	38.0	-0.6702
Root porosity (POR) (%)	34.9	39.7	26.1	26.5	34.9	0.5974
Chlorophyll-a (mg/g)	1.62	1.39	1.33	0.52	1.28	0.7357
Leaf surface area (cm^2)	59.7	24.1	95.7	53.2	46.5	-0.1828
Root dry biomass (g)	1.56	1.90	1.62	1.19	0.92	0.9134
Shoot dry biomass (g)	0.86	1.84	1.89	1.19	3.43	-0.3514
Leaf dry biomass (g)	1.84	6.87	3.08	2.90	1.64	0.6113
Root length (cm)	31.3	35.0	39.7	37.3	38.0	-0.5672
Shoot length (cm)	65.3	82.7	70.7	46.0	52.0	0.9377
Root number	41	26	43	50	95	-0.7279
Root diameter (cm)	0.1226	0.1216	0.1210	0.1321	0.1085	0.0202
Oxygen release rate ($\mu mol\ g^{-1}\ h^{-1}$)	328.4	364.4	286.3	196.4	212.3	1

[*] Oxygen release rate: the average value of all maximum oxygen release rates over 3 days. Correlation coefficients: Correlation coefficients between physical signs and oxygen release rate

When eco-physiological features influence root oxygen release, the physiological parameters interact with each other. If we choose only the main physiological factors through the correlation coefficients between each physical sign and oxygen release rate, without considering the combined action of all physical factors, the results may be inaccurate. The present study analyzed the relationships between two factors ignored other factors using partial correlation coefficient. *Table 4* displays the partial correlation coefficient between main factor and oxygen release rate. The results show that the partial correlation coefficient between leaf dry biomass and oxygen release rate (chlorophyll-a, shoot length, and POR were controllable factors) was $-0.206 < 0$, although the correlation coefficients between dry leaf biomass and oxygen release rate was higher (*Table 3*). This means that the higher correlation between dry leaf biomass and oxygen release rate depended on chlorophyll-a, shoot length, and POR. This is coincidental upon the mechanism of oxygen release from plants. Oxygen is produced through photosynthesis (Chen et al., 2008), transferred from the leaves to the roots of plants via pressurized convective transfer within the aerenchyma, and released to the rhizosphere. Chlorophyll-a, shoot length, and POR promoted the production, transport, and release of oxygen. Dry root biomass, POR, shoot length, and chlorophyll-a were the main influencing factors for root oxygen release.

Table 4. *Partial correlation coefficient between main factor and oxygen release rate*

Physical signs	Controllable factor	Partial correlation coefficient	Double factor correlation coefficient
Leaf dry biomass (g)	POR	0.482	0.6113
	Chlorophyll-a	0.648	
	Shoot length	-0.301	
	Root dry biomass	-0.206	
Root dry biomass (g)	POR	0.961	0.9134
	Chlorophyll-a	0.959	
	Shoot length	0.388	
	Leaf dry biomass	0.864	
Shoot length (cm)	POR	0.919	0.9377
	Chlorophyll-a	0.885	
	Root dry biomass	0.617	
	Leaf dry biomass	0.908	
POR (%)	Chlorophyll-a	0.299	0.5974
	Shoot length	0.419	
	Root dry biomass	0.637	
	Leaf dry biomass	0.461	
Chlorophyll-a (mg/g)	POR	0.592	0.7357
	Shoot length	0.419	
	Root dry biomass	0.782	

Figures 3–6 illustrate the fitting lines between root oxygen release rate and dry root biomass, POR, chlorophyll-a, and shoot length. The results revealed that root oxygen release rates are increased with increasing eco-physiological parameters such as dry root biomass, POR, chlorophyll-a, and shoot length.

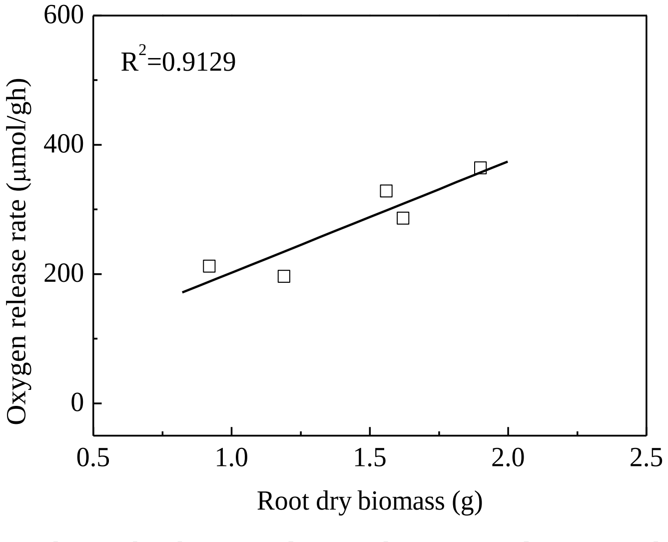

Figure 3. *Relationship between dry root biomass and oxygen release rate*

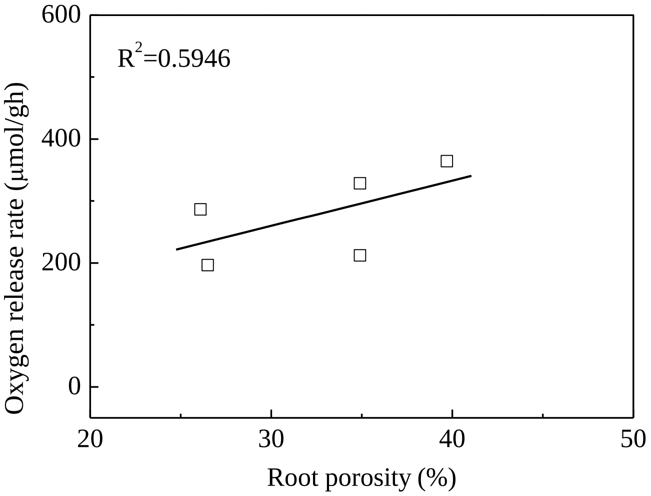

***Figure 4.** Relationship between root porosity and oxygen release rate*

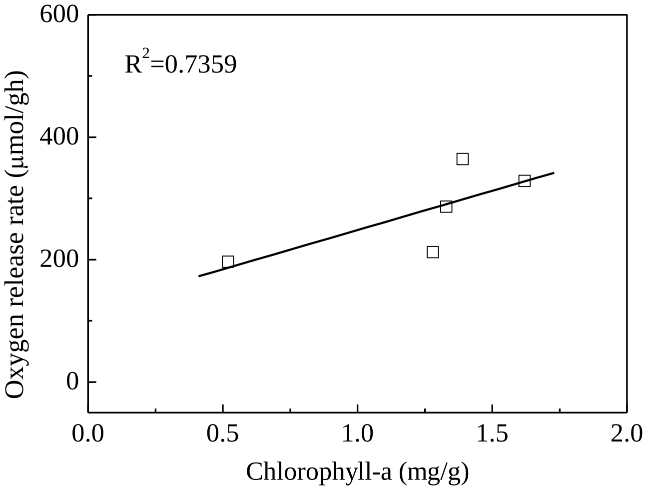

***Figure 5.** Relationship between chlorophyll-a and oxygen release rate*

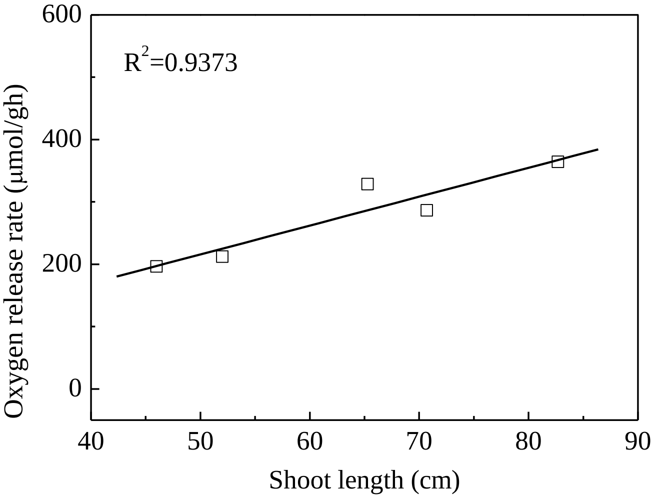

***Figure 6.** Relationship between shoot length and oxygen release rate*

Discussion

The data in the present study revealed that there were significantly different capacities of oxygen release among the five plant species in the same environment. *Typha orientalis* Presl. released less oxygen into the rhizosphere than *Phragmites australis*. These species belong to the Typhaceae and Gramineae, respectively. The characteristics of Gramineae and Typhaceae lead to the different capacity for radial oxygen release to the rhizosphere. A Gramineae herb is a monocotyledon, and perennial. The stem is hollow and nodal, and not solid. The leaves usually consist of a leaf and leaf sheath, and the leaf blade is flattened with a parallel vein. There is more chlorophyll-a in the leaf, and more below-ground biomass. A Typhaceae herb is aquatic and perennial, whose roots are rhizoid or consist of thick lateral roots. The stems and linear leaves are erect, and the vein is parallel. Oxygen release rates of Gramineae species are high, mainly due to its biomass, root physiology and morphological properties, as shown by Lai et al. (2011). Several species belonging to the Gramineae, such as *Arundo donax* var. *versicolor*, *Oryza sativa*, and *Panicum repens* have the capacity to release more oxygen to the rhizosphere because of higher porosity rates. There were significant positive correlations between rates of ROL and porosities (Mei et al., 2009).

The Gramineae plants had significantly higher porosity, which had a positive correlation with root oxygen release rates (*Fig. 4*). The transverse root structure of wetland plants comprised three parts: epidermis, vascular cylinder, and cortex. The cortex comprised the endodermis, mediopellis, and exodermis. In the mediopellis, there were varied sizes and patterns of cavities for different plants (Lai et al., 2011). The aerenchyma of the Gramineae plant roots were distributed radially between the vascular cylinder and the exodermis, and made up of large cavities forming from many small pores. Roots of this type are only present in aerenchyma area and are not affected by the number and size of vascular cylinder. The porosity was clearly higher.

Water levels can influence the porosity of all wetland plants. Sasikala indicated that a reduction state of media with a static water level promoted the formation of aerenchyma and POR (Sasikala et al., 2009). Water level fluctuation caused a considerable reduction in radial oxygen loss and POR. So we should control a well water level, which can make a reduced condition, to increase the POR.

The Gramineae plants generally had a higher biomass. Radial oxygen loss was positively correlated with total biomass, above-ground biomass ($r^2 = 0.86$) (Tanaka et al., 2007; Sasikala et al., 2009), root biomass of $D \leq 1$ mm, and leaf biomass (Lai et al., 2012). Salinity typically effects wetland plants on biomass production rates and allocation patterns (Sculthorpe, 1985; Morris and Ganf, 2001). In three emergent species, B. caldwellii, E. equisetina, and P. distichum, increases in height with increasing water depth (Johns et al., 2014). This growth response is typical of species (Baily-Serres and Voesenek, 2008).

The results of this study suggest that plant species had a significant influence on the capacity of plant oxygen release, and that we should choose Gramineae plants to create an aerobic environment. It is necessary to maintain a large biomass and enhanced porosity to improve the contribution of plants.

Previous results showed that the ability of wetland plants to release oxygen in CWs varied at different growing stages (Zhang et al., 2014). The plants examined in this study were mature. It is clear that, the research of more plant species is necessary to reach a more accurate conclusion.

Conclusions

The results of this study revealed a significant difference in the capacity of five wetland plant species in the same environment (*Acorus calamus* Linn., *Typha orientalis* Presl., *Thalia dealbata*, *Phragmites australis*, and *Pontederia cordata* Linn.) to release oxygen. *Typha orientalis Presl* released less oxygen into the substrate than did *Phragmites australis*. The maximum oxygen release rate of *Phragmites australis* ranged from 253.0 to 424.2 μmol g^{-1} h^{-1} over 3 days. The values of *Typha orientalis* Presl. ranged from 74.7 to 273.8 μmol g^{-1} h^{-1}. The root oxygen release rate was positively correlated with several eco-physiological and morphological features. The main influencing factors were dry root biomass, POR, shoot length, and chlorophyll-a.

The characteristics of Gramineae and Typhaceae lead to the different capacities in radial oxygen release to the rhizosphere. Gramineae species have higher oxygen release rates of root systems because of appropriate eco-physiological and morphological features, which include porosity, chlorophyll-a, and biomass. To create an aerobic environment we should choose Gramineae plants, because it is important to maintain large biomass and porosity to improve the plant contribution to oxygenation of the system.

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