



Short communication

Radial oxygen loss, photosynthesis, and nutrient removal of 35 wetland plants

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ABSTRACT

The aim of the present study was to test the correlation between radial oxygen loss (ROL), photosynthesis, and nutrient removal based on the hypothesis that ROL was principally a positive physiological process of wetland plants, and was correlated with photosynthesis and nutrient removal. Thirty five emergent wetland plants were used for the measurement of ROL, photosynthesis, and nutrient removal in micro-scale wetlands in a climate chamber. Significant differences among thirty five species were tested in ROL, photosynthetic rate, and nutrient removal rates. ROL was positively correlated with photosynthetic rate ($P=0.000$), transpiration rate ($P=0.005$), root activity ($P=0.000$), root biomass of $D \leq 1$ mm ($P=0.002$), above-ground biomass ($P=0.030$), leaf biomass ($P=0.023$), root porosity ($P=0.000$), maximum root length ($P=0.011$), and removal rates of TN and TP ($P=0.000, 0.002$), while negatively related to root biomass of $D \geq 3$ mm, and root longevity ($P=0.022, 0.007$). All the indices which were positively correlated with ROL, also positively correlated with plant growth. The results suggest that ROL may be an active physiological process or at least involves physiological processes of wetland plants. Significant differences existed among different wetland plants in ROL, photosynthesis, and nutrient removal, which should be considered in plant selection for constructed wetlands.

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1. Introduction

Oxygen is a key factor for wetland plants to live and to remove nutrients in anoxic conditions (Pezeshki, 2001; Stottmeister et al., 2003; Maltais-Landry et al., 2009). Wetland plants have better adaptation to anoxic environments, such as the aerenchyma, which is displayed by root porosity. Previous studies indicated that root porosity of wetland plants could account for up to 60% of root volume (Bedford et al., 1991; Chen et al., 2002; Li et al., 2011). Oxygen needed by wetland plants can be transferred from above-ground parts of the plant to below-ground parts through the aerenchyma (Justin and Armstrong, 1987; Brix, 1993). The phenomenon of wetland plant roots releasing oxygen through the aerenchyma to the rhizosphere is termed radial oxygen loss (ROL) (Armstrong, 1978). ROL is an important characteristic of wetland plants, which may relate to their adaptability to the wetland substrate (Armstrong, 1967; Stottmeister et al., 2003), and to the nutrient removal (Sorrell and Brix, 2003; Sasikala et al., 2009). The oxygen concentration

was higher in the rhizosphere, decreased away from the rhizosphere (Kirk et al., 1993; Van Bodegom et al., 2001), and formed an aerobic–anaerobic gradient with the distance away from the roots. This was beneficial to the growth of aerobic, facultative anaerobic and anaerobic microorganisms, and to the nutrient removal by the comprehensive effects of the microorganisms. ROL could also affect the redox potential of the wetland substrate (Kludze et al., 1993; Vartapetian and Jackson, 1997), therefore affecting many rhizosphere responses. For these reasons, the ROL of wetland plants has attracted many studies. However, the mechanism of ROL has not been clearly understood. The oxygen transportation within wetland plants was generally considered as physical processes, such as diffusion, and convective flow (Armstrong and Armstrong, 1991; Brix, 1993; Allen, 1997). Previous research has suggested that the physiological functions of shoots were of decisive importance for oxygen transfer within a wetland plant (Stottmeister et al., 2003). This suggests that ROL may correlate with the physiological activities of wetland plants. Therefore, it was difficult to understand the mechanisms of ROL without an intensive understanding of the correlation between ROL and the eco-physiological activities. However, less research has been done on the correlation between ROL and the eco-physiological activities, such as photosynthesis, root activity, and more works are urgently needed.

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Photosynthesis is an important physiological activity of plants, which directly reflects their growth and nutrient absorption. Photosynthesis produces oxygen, so it is feasible to ask whether photosynthesis of wetland plants correlates with their ROL. Some studies suggested that photosynthesis was the source of oxygen released by plant roots (Sand-Jensen et al., 1982; Connell et al., 1999). Wetland plants could transfer part of the oxygen produced in photosynthesis through the aerenchyma to the roots, and release it into the wetland substrate (Teal and Kanwisher, 1966; Brix and Schierup, 1990). However, research focusing on ROL and photosynthesis has, to date, been scarce, and it is still unclear how close a correlation exists between ROL and photosynthesis, and whether ROL is an active or passive process of wetland plants (Sorrell and Brix, 2003). We hypothesized that ROL was primarily an active process which was affected positively by photosynthesis, and correlated positively with nutrient removal. The objective of the present study was to investigate the supposition by comparatively studying the relationships between ROL, photosynthesis, and nutrient removal with a relatively large amount of species.

2. Materials and methods

2.1. Establishment of the small-scale constructed wetlands

Thirty five wetland plant species or varieties were used for the experiment (Appendix 1). The wetlands were established in circular plastic pails (23 cm × 19 cm × 23 cm, $D_1 \times D_2 \times H$). One young plant (about 20 cm in height, and about 10 g in weight) was planted in each wetland. The plants were fixed with round cystosepiments, which had a diameter about the same as the upper inner diameter of the pails. Each species-specific wetland had five replicates. All wetlands were arranged randomly at a distance 30 cm from each other in a climate chamber. The average daytime air temperatures (7:00–19:00) and evening temperatures were $28.0 \pm 1.0^\circ\text{C}$ and $18.0 \pm 1.0^\circ\text{C}$, respectively. Relative humidity was $80\% \pm 5\%$, and the illumination intensity was 20 Klx in daylight hours. The wetlands were filled with a nutrient solution. The plants were firstly cultured with tap water during the first week, then with a 50% nutrient solution added during the second week, and with a 100% nutrient solution added thereafter. Every wetland was provided with a 5.0 L solution, which was exchanged every 5 days, with an average hydraulic loading of $0.03 \text{ m}^3 \text{ m}^{-2} \text{ day}^{-1}$. The nutrient solution was prepared according to Wießner et al. (2005). The average concentration (mg L^{-1}) was 326 of chemical oxygen demand (COD), 62 of total nitrogen (TN), and 5.0 of total phosphorus (TP).

2.2. Root radial oxygen loss

ROL of the wetland plants was measured non-destructively during the day using the modified method of Armstrong and Wright (1975). The instruments used in the experiment included a polarograph, a cylindrical platinum electrode, an Ag/AgCl electrode, and a list-style recorder. The measurements were conducted during the 10–11th week of the study. Young roots of about 9–11 cm in length and with a $D < 0.15$ cm from each wetland were measured at 0.5 cm from the root tip, with the other part of the root being dipped in the original nutrient solution. Measurement of the electric current was started as soon as a balanced voltage was set. The ROL was calculated using the formula: $\text{ROL} = nFA/i$, where ROL is the external O_2 flow rate ($\text{ng cm}^{-2} \text{ min}^{-1}$); i is the electric current showed in the polarograph (μA); n is the number of electrons for deoxygenating one molecular oxygen, set at 4; F is Faraday's constant 96,500; A is the surface area of the root within the cylindrical platinum electrode (cm^2).

2.3. Photosynthetic rate and other relative indices

Net photosynthetic rate, transpiration rate, and stomatal conductance were measured using a LI-6400 portable photosynthesis measurement system simultaneously during the 10–11th week when ROL was measured. Five leaves from each plant were measured, and each assay was performed in triplicate to obtain the average.

2.4. Root activity

Plant roots of 1–2 g, with a $D < 3$ mm, were sampled from each wetland during the 12th week for root activity measurement. Root activity was determined using the method of Yang et al. (2004).

2.5. Biomass

At the end of the experiment, the plants were harvested. The dry weight of the biomass of shoot, roots of $D \leq 1$ mm, $1 \text{ mm} < D < 3$ mm, $D \geq 3$ mm, and rhizomes was weighed to an accuracy of 0.01 g after being dried to a constant weight at 80°C .

2.6. Nutrient removal

Nutrient removal (%) was measured during the 14th week. About 300 mL of nutrient solution was taken for measurement from each wetland after 48 h of retention. Concentrations of TN, TP and COD were measured according to the standard method (State Environmental Protection Administration of China, 2002). Nutrient removal (%) was calculated on an influent basis.

2.7. Statistical analysis

The mean, standard deviation and correlation analysis of the parameters were calculated using SPSS and Microsoft Excel 2003. One way ANOVA and the LSD test was used to test the statistically significant differences between different plants.

3. Results

3.1. Root radial oxygen loss and root activity

Significant differences in ROL existed among the 35 plant species ($P = 0.000$). The highest average ROL ($\text{ng cm}^{-2} \text{ min}^{-1}$) was 151 from *Caldesia reniformis*, second being 150 from *Cyperus flabelliformis*, 146 from *Sagittaria trifolia*, and 139 from *Lythrum salicaria*. *Colocasia tonoi* exhibited the lowest ROL. Thirteen species exceeded 100 in ROL, while 17 species ranged from 50 to 100, and 5 species had an ROL below 50.

There were significant differences among the 35 plant species in root activity ($P = 0.000$). *Eclipta prostrata* showed the highest root activity ($193 \text{ mg g}^{-1} \text{ DW h}^{-1}$). There were 4 species exceeding 100 in root activity, 18 species ranging from 50 to 100, and 13 species exhibiting a root activity below 50.

3.2. Photosynthetic rates and relative indices

Photosynthetic rates were also significantly different among the 33 plant species ($P = 0.000$) (photosynthetic rates of 2 species could not be tested because of metamorphosis of their leaves). The highest photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was 12.20 from *Canna indica* and *Pontederia cordata*, compared to the lowest of 2.30 from *C. tonoi*. There were 4 species exceeding 10 in photosynthetic rates, 9 species ranging from 8 to 10, 10 species ranging from 6 to 8, 9 species ranging from 4 to 6, and 1 species measuring below 4.

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