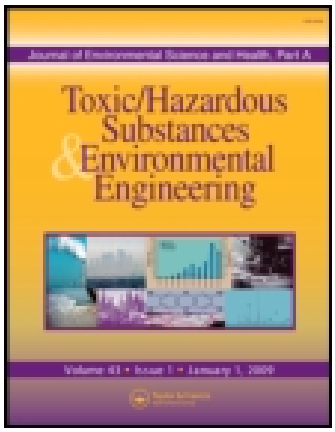

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Diurnal fluctuations in oxygen release from roots of Acorus calamus Linn in a modeled constructed wetland

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Diurnal fluctuations in oxygen release from roots of *Acorus calamus* Linn in a modeled constructed wetland

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Oxygen is known to be released from plant roots, but has seldom been quantified for wetland plants. Our study aims to quantify oxygen release from the roots of one wetland species in China, and use this knowledge as a basis for future modeling. We measured diurnal fluctuations in oxygen release from the roots of *Acorus calamus* Linn in a modeled constructed wetland (CW) using a titanium (III) citrate buffer. Oxygen release was monitored every two hours. Maximum oxygen release was recorded in the range of 215.2–750.8 μmolg^{-1}h^{-1} and occurred around 15:00. The maximum value of photosynthetically active radiation (PAR) was in the range of 1281.8–1712.0 mmolm^{-2}s^{-1} and occurred around 13:00. Both the oxygen release rate and PAR were found to approach zero at night. Our results indicate that oxygen release depends largely on light intensity and exhibits a diurnal periodicity with release occurring only during daytime. Rate of root oxygen release varied during the daytime and this temporal variation was well described by the Gaussian function. While further validation is needed, we suggest that the Gaussian function may be used as the basis for modeling root oxygen release in natural and constructed wetlands.

Keywords: Wetland plants, constructed wetlands, diurnal fluctuation, photosynthetically active radiation, oxygen release rate, Gaussian function.

Introduction

Constructed wetlands (CW) are one method of low-cost wastewater treatment. They have been the focus of increasing international interest because of their water treatment efficiency and their natural appearance. Aerobic decomposition of organic matter including nitrification, is dependent on oxygen within a wetland, and is a key process within a CW. Oxygen can enter the wetland in three ways: as dissolved oxygen in the inflow liquid, as direct surface reaeration from the atmosphere, and as root release from macrophytes. Plants in natural and constructed wetlands are known to transport oxygen to their roots and release it into their root zones.[1] Rhizosphere oxidation activates biochemical reactions and biological processes including degradation of organic compounds and nitrification by rhizospheric microorganisms.[2] Although oxygen is known to be released from the root systems of plants, the extent has not been quantified.[3]

It is recognized that the rate of oxygen release by plant root systems varies in accordance with environmental factors.[4] These include rhizospheric characteristics such as redox state, pH, oxygen levels, and chemistry, and plant characteristics such as plant mass, species, and stage of development, as well as climate. Other factors have been reported. For example, Jespersen et al.[5] studied the effect of surrounding sediment on the oxygen release rate of roots, by comparing *Typha* plants grown in two sediments (a natural organic sediment and an acetate-enriched sediment). Oxygen demand in the acetate-enriched sediment was higher than that in the natural sediment.

The sediment type influenced growth pattern and root shape, and thus the oxygen release rate. Sasikala et al.[6] investigated the effects of water level fluctuations on radial oxygen loss (ROL) of plants in a CW subjected to vertical subsurface flow. They found that the quantity of oxygen released by root systems could be significantly reduced by fluctuating the water level. Stottmeister et al.[4] described how gas transport from above-ground sections of the plant to the roots was aided by tissue known as aerenchyma

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that forms within the rhizomes and roots of submerged plants.

The dominant process of oxygen release is, or course, photosynthesis; most oxygen transported to roots is of photosynthetic origin.[7] Oxygen is transferred from leaves to roots by molecular diffusion and convection. Changes in photosynthetic rate in response to environmental factors directly influences oxygen-production and transportation; thus ultimately affecting wetland function.[8] Oxygen release rates are known to be several times greater in the light than in the dark.[7] Effects of weather conditions on oxygen release rate need elucidation; this would enable improved design and operation of aquatic, plant-based, wastewater treatment systems.[2] The influence of light intensity on oxygen release needs to be examined in detail, as do diurnal patterns in release rate.

We used young *Acorus calamus* plants to investigate the effects of light intensity on oxygen release and diurnal patterns in release rate, We modeled a constructed wetland using a titanium (III) citrate buffer, We developed a model based on a Gaussian distribution to describe observed root behavior. This model enables prediction of diurnal fluctuation in root oxygen release.

Materials and methods

Experimental materials and procedures

Young *Acorus calamus* plants were collected from a natural wetland located on the shores of Xuanwu Lake, Nanjing, China. These were planted in individual plastic pots filled with purpose-made nutrient solutions (with average concentrations of chemical oxygen demand (COD) at 50 mg/L and total nitrogen (TN) at 15 mg/L) for 3 weeks. The plants were then removed from pots and their roots gently washed free of debris. All plants had 20–40 adventitious roots of 12–21 cm in length, and up to 0.087 ± 0.029 cm in diameter. Several plants had around 118 adventitious roots. Plants were 41–58 high.

Oxygen release from the roots was examined using a non-phytotoxic, titanium (III) citrate buffer; allowing measurements of oxygen release in a reducing, oxygen-scavenging solution with low redox potential.[9,5] A 1000 mL jar was filled with 900 mL of distilled water. The water was sparged with N_2 gas for 60 min to remove dissolved oxygen, and N_2-sparging was continued while titanium (III) citrate stock solution (0.2249 g citric acid, 8 mL $TiCl_3$) was added. Sparging also ensured that the buffer was well mixed. The roots of the *Acorus calamus* plants were then submerged in the solution. A layer of paraffin oil (5 mm) was poured on top of the solution to prevent re-aeration from the atmosphere; thus the roots were the only possible oxygen-source within the chamber. The basal part of each shoot had previously been wrapped with tinfoil to prevent paraffin from infiltrating the aerenchyma. The root chamber was shielded from light using a tightly fitting tin foil cover. Jars without plants were prepared as negative controls. Figure 1 shows this experiment. Jars were in open air and natural light. Light intensity was measured in lux every hour using a luminometer (MODEL ZDS-10F-2D). Experimental variables are shown in Table 1. After the incubation, dry weights of plant roots were measured after drying for 24 h at 105°C.

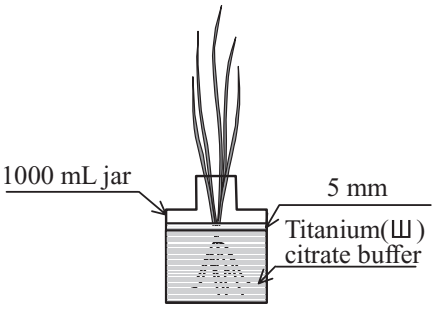

Fig. 1. Schematic diagram of device for detection of root oxygen release.

Sampling and analytical methods

Since oxygen released from roots is oxidized by Ti^{3+} in the titanium (III) citrate buffer, rates of root oxygen release can be calculated from the rate of decrease in Ti^{3+} concentration in the jars. The brown titanium (III) citrate solution was observed to gradually become clear during oxidation. Samples were taken every hour using a small syringe. Absorbance at the wavelength of 527 nm was measured immediately using a spectrophotometer. Absorbencies were compared with those of solutions with a known Ti^{3+} concentration. Light intensity, temperature and humidity of the surrounding air were measured concurrently. The reaction between Ti^{3+} and O_2 is shown in Equation 1.[10] It can be seen that 1 mole of O_2 is consumed when 4 moles of Ti^{3+} are reduced. Oxygen consumption (ΔO_2, mg) was thus calculated using Equation 2.

$$O_2 + 4Ti^{3+} + 4H^+ = 4Ti^{4+} + 2H_2O \quad (1)$$

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

Table 1. Light intensity, temperature and humidity during experiments.

Experimental date	*PAR* ($\mu mol \cdot m^{-2} \cdot s^{-1}$)		*Temperature* (^oC)		*Humidity* (%)	
	Average	*range*	*Average*	*range*	*Average*	*range*
22 April 2009	444.2	0–1281.8	24	19–28	38	15–61
25 April 2009	501.1	0–1536.4	22	18–25	27	10–52
26 April 2009	630.5	0–1712.0	22	18–25	27	9–55

*Sample number is 24. PAR is photosynthetically active radiation. One lux is 0.019 $\mu mol \cdot m^{-2} \cdot s^{-1}$.[12]

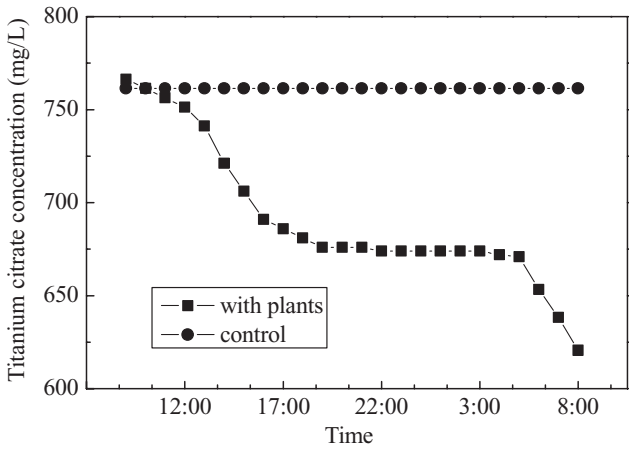

Fig. 2. Diurnal fluctuations in titanium (III) citrate concentration.

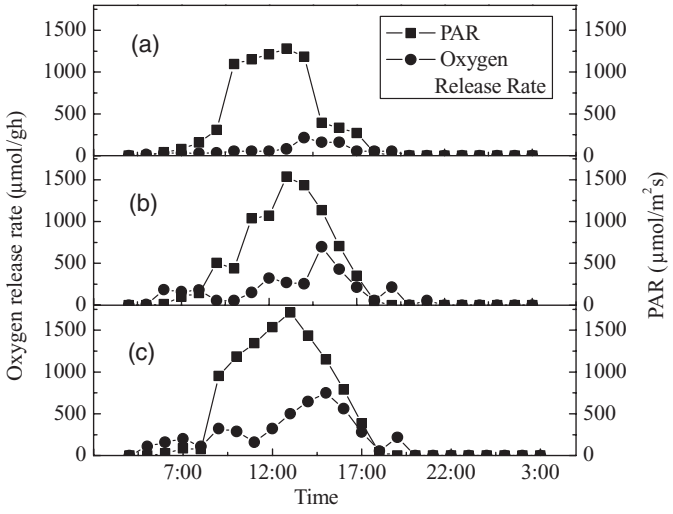

Fig. 3. Diurnal fluctuation in root oxygen release: (a) 22nd April; (b) 25th April; (c) 26th April 2009.

where V is the volume of titanium (III) citrate buffer (0.9 L), C_0, and C_e are the initial and end Ti^{3+} concentrations. The root oxygen release rate (V_O, $\mu molg^{-1}h^{-1}$) was calculated using Equation 3.

$$V_O = \frac{\Delta O_2 \times 1000}{24 \times 32 \times \text{Root dry weights}} \quad (3)$$

Diurnal fluctuations in oxygen release from roots of *Acorus calamus* Linn could be described using a Gaussian function. First, fit the data of the light intensity and oxygen release rate using the Gaussian function, respectively. Second, obtain the relationships between Gaussian function parameters of light intensity and that for oxygen release rate. Third, calculate the oxygen release rate using the light intensity data for other days. Last but not least, the experiments were further conducted in October 2009 to validate the proposed model based on the Gaussian function, and PAR was tested every hour during the daytime (4:00–20:00).

Results

Oxygen release rate of plant roots

Figure 2 displays a typical daily change in titanium (III) citrate concentration. Concentration of titanium (III) citrate in the control jars did not change during the experiment. This suggests that variation in Ti^{3+} concentration was caused by oxygen released by plants in the planted jars.

Values for root oxygen release were obtained from Ti^{3+} concentrations measured in the test jars, via the above equations. Diurnal fluctuations in oxygen release and PAR are shown in Figure 3. Our results reveal a significant difference in rate of root oxygen release between day and night. Oxygen release increased gradually with increasing light intensity during the morning. A decrease in oxygen release occurred during the decreasing light intensity of the afternoon. At night, oxygen release rate approached 0 $\mu molg^{-1}h^{-1}$. In all three experiments, the start and end times of oxygen release were closely related to light. The maximum oxygen release rate (215.2–750.8 $\mu molg^{-1}h^{-1}$) was observed during the daytime at 15:00 hrs, while the maximum light intensity was observed at 13:00 hrs. The maximum value of PAR ranged from 1281.8 to 1712.0 $mmolm^{-2}s^{-1}$. Clearly, the peak of root oxygen release occurred after the peak of light intensity.

Diurnal fluctuations in root oxygen release and light intensity—application of a Gaussian function

Daily fluctuations in root oxygen release are schematically summarized in Figure 4. Two time intervals were used during the diurnal cycle; t_{Ls} and t_{Le} signify the start and end times of the bright period. These also correspond to sunrise (t_{Ls}) and sunset (t_{Le}). L_{max} is the maximum light intensity at the corresponding time t_{Lmax}. t_{Os} and t_{Oe} signify the

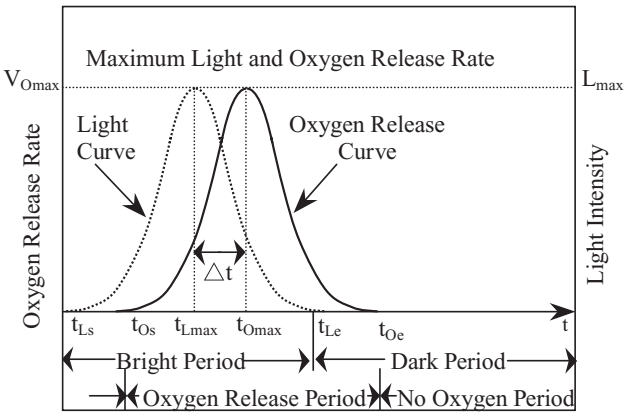

Fig. 4. Schematic diagram of diurnal fluctuation in root oxygen release.

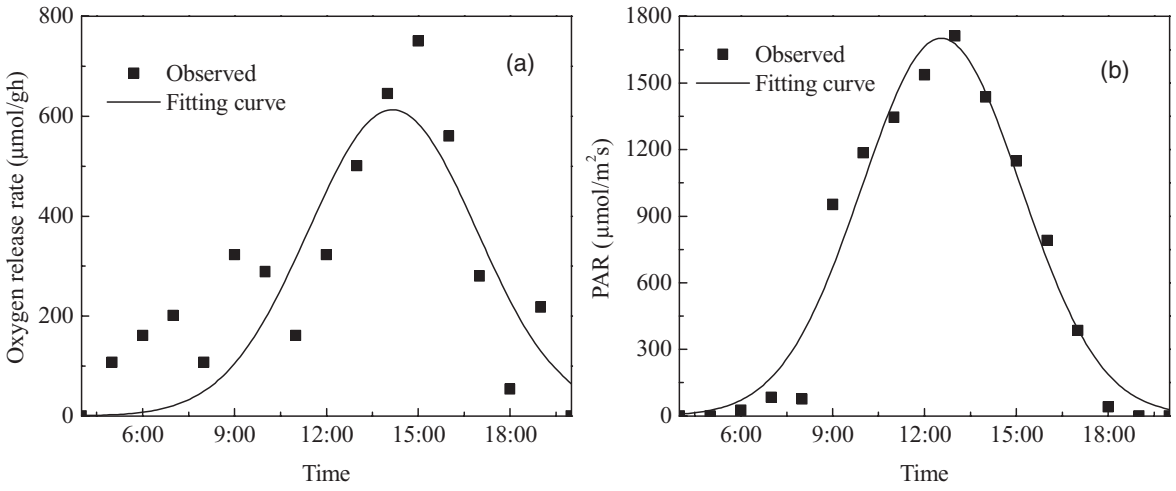

Fig. 5. Fitting root oxygen release (a) and light intensity (b) using the Gaussian function.

start and end times of the oxygen release period. V_{Omax} is the maximum oxygen release rate at the time of t_{Omax}. Since the time of maximum oxygen release occurred after the maximum light intensity, the time difference between these, termed lag time (Δt), is also defined. This time lag may be caused by photosynthesis and consequent oxygen transport through the aerenchyma.

To quantify diurnal fluctuations in root oxygen release, experimental data was preliminarily fitted using several functions (t_{Os} was 4:00 and t_{Oe} was 20:00). The best fit was achieved using the Gaussian function[14]; this represents a unimodal distribution (Figure 5a). The goodnesses of fit (R^2) were 0.7574, 0.5357, and 0.6796 with a 95% confidence interval, on the 3 days studied. Based on the Gaussian function, diurnal fluctuation in root oxygen release can described as

$$V_O = ae^{-\frac{(t-t_{Omax})^2}{c^2}} \quad (4)$$

where t is time (4:00–20:00); a is the maximum rate of oxygen release in a day; and c expresses the gradient of the Gaussian function for rate of oxygen release. A small value of c indicates a steep Gaussian function, while a large value of c gives a gradually varying Gaussian function. Figure 5a shows root oxygen release data from one day with the Gaussian function fitting; where a, c and t_{Omax} are estimated to be 613.1 $\mu molg^{-1}h^{-1}$, 3.884 and 15:00, respectively.

Light intensity data during the daytime (4:00–20:00) also follow a Gaussian function (Fig. 5b). This can be described as

$$PAR = be^{-\frac{(t-t_{Lmax})^2}{d^2}} \quad (5)$$

Where *PAR* is photosynthetically active radiation in $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$; *b* is the peak value of *PAR* in a whole day; and *d* is the gradient of the Gaussian function for light intensity. Figure 5b shows the light intensity data on one day with the Gaussian function fitting, where *b*, *d* and t_{Lmax} are estimated to be 1702 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$, 3.672 and 13:00, respectively.

The peak rate of root oxygen release was observed 2 h after the maximum light intensity. The correlation between light intensity and oxygen release was analyzed (Fig. 6). It is evident that root oxygen release was influenced dramatically by light intensity. The oxygen release rate increased exponentially with increased *PAR* ($R^2 = 0.8689$):

$$V_O = 62.22e^{0.00138PAR} \quad (6)$$

By combining Equations 4, 5 and 6, the following equation was obtained:

$$ae^{-\frac{(t-t_{Omax})^2}{c^2}} = 62.22e^{0.00138\left(be^{-\frac{(t-t_{Lmax})^2}{d^2}}\right)} \quad (7)$$

In Equation 7, $t_{Lmax} = t_{Omax}$ because the oxygen release curve (see Figure 4) shifted to 2 h later, following the

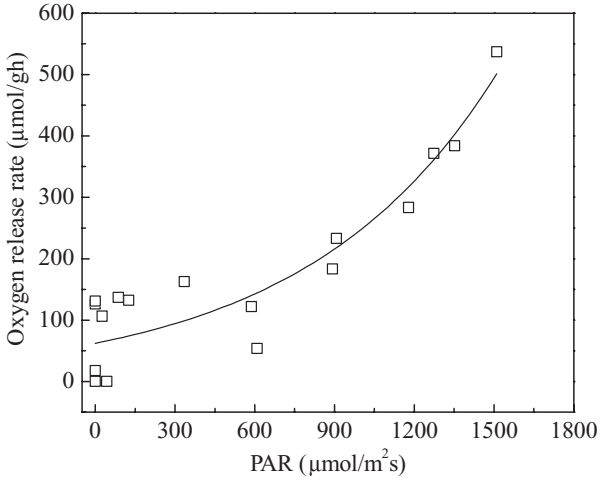

Fig. 6. Effect of light intensity on root oxygen release.

Table 2. Modeling parameters.

	PAR		*Oxygen release*	
Date	*b* ($\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$)	*d*	*a* ($\mu molg^{-1}h^{-1}$)	*c*
2^{nd} October	1214	3.137	332.31	3.020
3^{rd} October	1092	3.33	280.82	3.317
6^{th} October	1254	3.155	351.16	3.047

correlation of light intensity and oxygen release data, as described in Equation 6.

If the time (t) happened to be equal to the peak time (t_{Omax} or t_{Lmax}), Equation 7 would become

$$a = 62.22e^{0.00138b} \quad (8)$$

Equation 8 indicates that parameter a for describing oxygen release behavior is related to b in the light intensity equation by an exponential function.

The relationship between parameters c and d also follows an exponential function. Values of c and d derived from our experiments were fitted using an exponential function ($R^2 = 0.9587$). The relationship is as follows:

$$c = 0.66e^{0.4856d} \quad (9)$$

Based on the above results, the Gaussian function can be used to predict oxygen release rate by the following procedure: (i) obtain light intensity data; (ii) fit the data using the Gaussian function from which parameters b and d can be obtained using Equation 5; (iii) determine the values of a and c using Equation 8 and Equation 9, respectively; and (iv) calculate the oxygen release rate using Equation 4.

Validation

Modeling parameters obtained are shown in Table 2. Oxygen release values and corresponding predictions using the Gaussian function are presented in Figure 7. Our model data closely match our experimental values. From the results of this simulation, it can be seen that the Gaussian function can be satisfactorily used to predict diurnal fluctuations in oxygen release by roots of wetland plants.

Discussion

In this study, the oxygen release rate from the roots of a wetland plant, *Acorus calamus* was examined. We have shown that oxygen release rates for *Acorus calamus* (Fig. 3) appear to be much higher than those reported for other wetland plant species.[11,12] An oxygen release rate of 7.40–13.24 $\mu molO_2\ h^{-1}g^{-1}$root dry weight (dw) was reported for the rice varieties 'Shengtai' and 'Suyunuo,[12] while an oxygen release rate of 1.6 $\mu molO_2h^{-1}g^{-1}$dw was reported for the sedge *Cladium*.[11] Reported oxygen release rates may be significantly different even for the same wetland

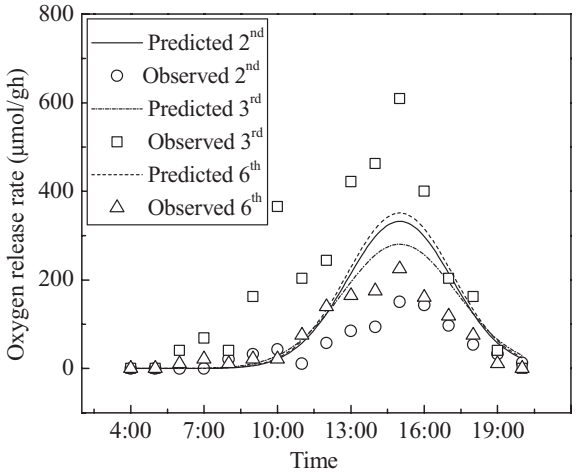

Fig. 7. Comparison of observed and predicted oxygen release rates in October 2009.

plant. Sorrel and Armstrong,[9] for example, reported an oxygen release rate of 126 $\mu molO_2h^{-1}g^{-1}$dw for *Juncus ingens,* while a value of 1.5 $\mu molO_2h^{-1}g^{-1}$dw was reported by Chabbi for the same species.[13] This variation may be partially attributable to testing conditions, including varying light intensity.

Oxygen is produced during photosynthesis[14] and transferred from leaves to roots through the gas-filled tissues of a plant by a process of diffusion and convection.[15] Oxygen is then released to the rhizosphere by gas exchange. We found that photosynthetic rate is highly correlated with light intensity. Photosynthetic parameters affect a plant's ability to produce oxygen.[8] The light-dark switch generates a large and rapid fluctuation in the internal oxygen levels of plants.[15] Thus, plants also exhibit great fluctuations in released oxygen. We show that rate of oxygen release depends largely on light intensity and exhibits a diurnal periodicity. Variations in oxygen release and light intensity follow unimodal patterns during the daytime and can be accurately described by the Gaussian function. The maximum root oxygen release was measured 2 h after the maximum measured light intensity (Fig. 3). From this we established the relationship between root oxygen release and light intensity. A recent study has shown that maximum root oxygen release (with up to 35% oxygen saturation at the root surface) for *Myriophyllum spicatum* occurred under light conditions, while a decrease of about 30% was observed in the dark.[7] Our study gives a detailed profile of diurnal fluctuations in root oxygen release correlating with natural light.

Our study also presents a methodology for quantifying root oxygen release using the Gaussian function. This allows use of light intensity data in prediction of the quantity of oxygen likely to be released. Further studies are needed to demonstrate the applicability of the Gaussian function for other wetland plants. It should be noted that our method and predictions are based on experimental data collected at

Nanjing; this area has a unique climate (temperate). Studies of other plant species, in other climates and differing natural light conditions should be considered before the methodology is applied more generally.

Conclusions

The oxygen release rate of wetland plants exhibits diurnal fluctuations. Light intensity is a major factor influencing oxygen release. During the morning, oxygen release rate increased with increasing light intensity. Values for both oxygen release and light intensity decreased gradually during the afternoon; and approached 0 μmolg^{-1}h^{-1} at night. Fluctuation in root oxygen release and light intensity follow a unimodal distribution. The Gaussian function is demonstrated to accurately describe the observed daytime variation in root oxygen release and may be used to predict root oxygen release in constructed wetlands.

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