Diurnal fluctuation of root oxygen release rate and dissolved oxygen budget in wetland mesocosm

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Abstract

To assess the role of plants for oxygen offering in constructed wetlands, this study

DO is dissolved oxygen, CW is constructed wetlands, HLR is hydraulic load rate, PAR is photosynthetically active radiation.

experimentally evaluated the amount of root oxygen release rate using mass balance method. The mass balance calculation is based on the following components: respiratory oxygen consumption of the roots; oxygen required for degradation of the organic matters; oxygen required for nitrification; and the amount of dissolved oxygen in the influent, effluent and substrate water. Experimental results have demonstrated that the root oxygen release rate was ranged from 20.3 to 58.3gO_2/m^2.d with average value of 38.4gO_2/m^2.d, which was affected dramatically by light intensity. Only 35% and 9% of the oxygen released by roots were used in the degradation of organic matters and nitrogen-nitrification, respectively, while 56% was used for roots respiration with little to be released to the surrounding medium. The results also showed that diurnal fluctuation of oxygen release and light intensity followed unimodal distribution. Meanwhile, a better understanding of the DO (dissolved oxygen) budget was proposed. Root oxygen release rate could be described by two fractions. One is “net specific oxygen release rate” and the other is “dischargeable oxygen release rate”.

Keywords: Constructed wetlands; Diurnal variation; DO budget; Mass balance method; Oxygen release rate

1. Introduction

The global use of constructed wetlands (CW) as an eco-friendly, low maintenance, low-cost, and efficient alternative to conventional wastewater treatment has been growing over the last decades [1,2]. Pollutants are reduced from wastewater in the CW

by a variety of physiochemical and biological processes. Available oxygen in CW is an important factor in degradation of organic matter and transformation of ammonium-nitrogen, both of which are oxygen limiting processes. When hypoxia (dissolved oxygen <2mg/L) occurs, a wide range of negative effects on aquatic ecosystems and organisms could be caused [3].

Coupled nitrification-denitrification is considered as the main N sink in CW [4]. Nitrification is an aerobic chemoautotrophic process while denitrification is an anaerobic heterotrophic microbial process. The nitrification step represents the main limiting factor for N removal in CW because of low oxygen availability in most cases [5,6]. Low oxygen content results in low aerobic organic matter decomposition [6,7]. In order to enhance N removal efficiency, oxygen must be provided to the nitrifying microbes through oxygenation of the wetland matrix via several means. Plants provide oxygen to the rhizosphere via passive or active oxygen transport through their stems from the atmosphere to the roots resulting in higher N removal rates in planted CW [8].

Recently, the interest in the main term of oxygen is increasing. Many studies have been conducted to illustrate the mechanisms responsible for mass balance of DO (dissolved oxygen) in estuarine and coastal areas worldwide [9]. However, there is little detailed information about the oxygen mass balance in CW. Furthermore, only few attempts have yet been made to evaluate the diurnal changes of oxygen consumption during degradation of organic matter or nitrogen oxidation. Accordingly, little was known about the diurnal variation of oxygen release by the roots of wetland plant. The role of plants for oxygen offering remains unclear. Therefore, the objective

of the present study was to provide a better understanding of the DO budget and the daily variation of the root oxygen release rates, which were explored based on mass balance method.

2. Materials and methods

2.1. Experimental mesocosm

The model wetland used in this study was a sub-surface vertical flow CW. Mesocosm was prepared from perspex column of 150mm in diameter and 1000mm in height. The mesocosm was filled with 2-5mm sized scoria as the substrate in a depth of 900 mm, giving an average porosity value of 0.46. Four sampling ports (S1, S2, S3, S4), inlets and outlets at different heights of the mesocosm (model CW) were setup (Fig. 1). Young *Acorus calamus* Linns collected from a natural wetland located in Xuanwu Lake, Nanjing, China, were planted in the wetland mesocosm. It was exposed to the open air with natural light outside the laboratory building.

Ammonium chloride, potassium dihydrogen phosphate and glucose were used as the sources of ammonium and organic matters. They were mixed with tap water to prepare the artificial wastewater [10]. The characteristics of the wastewater were as follows: TN 15.1mg/L (ranged between 12.0-18.7mg/L), NH_4^+-N 13.6 (ranged between 12.1-15.2 mg/L), NO_3^--N 1.7 mg/L (ranged between 1.5-1.8 mg/L) and NO_2^--N 0.006 mg/L (ranged between 0-0.011 mg/L). The wastewater was pumped into a holding tank, from where the mesocosm was supplied. Hydraulic load rate (HLR) was 0.16m^3/m^2 d, and the hydraulic retention time was about 2.43d. The model CW

was operated for one year. When the mesocosm reached its steady state after the start-up period, Samples of influent and effluent were collected from the six sampling points along the mesocosm every two hours. The samples were then analysed for DO, BOD_5, total nitrogen, ammoniacal-nitrogen, nitrite and nitrate according to the Standard Methods for Examination of Water and Wastewater [11]. Light intensity was measured every two hours using a luminometer (MODEL ZDS-10F-2D). The unit of light intensity is lux. In this paper, light intensity was described as photosynthetically active radiation (PAR). One lux is 0.019 $\mu mol \cdot m^{-2}\ s^{-1}$ [12].

2.2. Respiration of wetland plants

To prepare the examination, the surface of substrate (scoria) was covered with vaseline to prevent re-aeration from the atmosphere. The plants were covered with a black plastic bag to prevent photosynthesis. These were to make sure that the respiration was mainly taking place in the substrate. For the purpose of examining the respiration of wetland plants, the oxygen in the blank influent (without pollutants) of the wetland mesocosm was carefully pre-monitored. Such the oxygen was then absorbed by the root systems of plant in the process of respiration. Two hours later, the oxygen in effluent was examined. The difference of oxygen (in mass) can be used to calculate respiration rate.

The difficulty of the examination is the disturbance of oxygen from the air. The details of the procedure were as follow: Firstly, the volume of the blank influent was carefully controlled and the DO concentration was pre-monitored. Secondly, before the

blank influent was introduced to the mesocosm, the O_2 in the pore space of the substrate was replaced by N_2. The influent was then put into the mesocosm suddenly. Two hours later, the mesocosm was carefully drained using a vacuum-pump. At the same time, N_2 was injected into the mesocosm to prevent re-aeration. Finally, the volume and DO concentration of the effluent of the wetland mesocosm was examined, this allows to calculating the O_2 mass.

2.3. Statistical analyses

2.3.1. Oxygen consumption rate calculations.

The reasonable way to calculate the DO consumed by bacteria is via the decomposition of the organic matters [13]. Organic pollutants in wastewater are adsorbed on microbial cell surface which inhabits on the substrate surface. Pollutants are metabolized by microbes under catalysis of various enzymes. Oxygenolysis of partial organic matter by microbes is called catabolic metabolism. The other organic pollutants are taken in by microbes, and converted to new cells, this process is called anabolism. Based on these two metabolic processes, the oxygen demand of reducing organic matter ($O_{organic}$, mg/L) was analyzed theoretically as follows [13, 14]:

$$O_{organic} = a' BOD_r + b' P \quad (1)$$

Where the first part is the oxygen consumed in the process of catabolic metabolism. The second part is the oxygen consumed in the process of anabolism. a' is oxygen demand for the complete degradation of 1kg BOD_5; BOD_r is the removal of

BOD_5 in substrate, mg/L; b' is the oxygen demand of active biofilm per unit weight; P is the quantity of active biofilm which adsorbs to every 1 m^3 substrate (kg/m^3). Oxygen consumption rate (gO_2/m^2d) could be calculated as multiplying the oxygen demand by hydraulic load rate ($0.16m^3/m^2$ d). It is noted that Ye [13] reported that $b'P$ value was to be 0-0.00042kg/m^3 at different depths. Therefore, $b'P$ could be ignored. Here, the value of a' is 3.76 ($kgO_2/kgBOD_5$) since 3.53kg glucose is corresponding to 1$kgBOD_5$ while 1kg glucose is corresponding to 1.07kg oxygen.

Oxygen consumed by nitrification was calculated based on Eq. (2) and Eq. (3). Oxidizing 1g of NH_4^+-N and NO_2^--N requires 3.43g and 1.14g of oxygen, respectively.

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \quad (2)$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \quad (3)$$

2.3.2. Oxygen mass balance calculations.

In CW mesocosm, the root oxygen release could be assessed with mass balance method [15]. The main sources of oxygen in the substrate water include the inflow carrying, aeration and oxygen releasing from roots of plant. Output of oxygen includes outflow carrying, nitrification consumption and organic mater consumption. Mass balance equation of oxygen is given below:

$$O_{plant} = (O_{out} + O_{organic} + O_{nitrification} + O_{substract} + O_{res}) - O_{in} \quad (4)$$

Where O_{plant} is root oxygen release, $gO_2m^{-2}d^{-1}$; O_{out} is outflow carrying, $gO_2m^{-2}d^{-1}$; $O_{organic}$ is organic matter consumption, $gO_2m^{-2}d^{-1}$; $O_{nitrification}$ is nitrification consumption, $gO_2m^{-2}d^{-1}$; $O_{substract}$ is DO in substrate water, $gO_2m^{-2}d^{-1}$; O_{res} is

156 respiration consumption, $gO_2m^{-2}d^{-1}$; O_{in} is inflow carrying, $gO_2m^{-2}d^{-1}$.

157

158 *2.3.3. Prediction of root oxygen release.*

159 The root oxygen release rate in CW can be predicted using Gaussian function [16].
160 The light intensity, which was examined simultaneous with the water sample, can be
161 used for prediction purpose.

162

163 **3. Results**

164 Variations of oxygen consumption rate are illustrated in Fig. 2. It shows that the
165 oxygen consumption fluctuations for organic matter degradation and nitrification are in
166 the range of 3.1-26.1 $gO_2/m^2.d$ and 0.8-7.6 $gO_2/m^2.d$, respectively. More importantly,
167 the results have revealed a significant difference in the oxygen consumption rate
168 during day and night. Oxygen consumption rate was higher during daytime, following
169 an unimodal distribution pattern. The removal of organic matter and nitrogen is
170 accordingly followed the same pattern as oxygen consumption.

171 Fig. 3 illustrates the DO concentration of the water samples in the CW mesocosm,
172 which was calculated by combining several DO concentrations at different depths. The
173 CW mesocosm was divided into 5 units from the bottom at 0cm, 20cm, 45cm, 60cm,
174 and 75cm, respectively. The DO concentration and the volume of every unit were used
175 to calculate the total mass. The figure reveals that DO in the water changed in two
176 “day-night” cycle. In substrate water DO levels fluctuated, ranging from 0.03-0.39, due
177 to the oxygen released by root systems of wetland plant. Relatively, the inflow DO

concentration was stable. DO levels were dropped significantly in CW mesocosm, from an average of 0.9mg/L in influent to 0mg/L in effluent.

Fig. 4 illustrates the daily changes of respiration rate by the roots of plant. Root respiration measured as O_2 consumption in CW system is generally followed a daily cycle. Respiration rate is ranged from 14.4 $gO_2/m^2.d$ to 30.3 $gO_2/m^2.d$. Temperature is ranged from 15℃ to 35℃. The respiration rate is increased with increasing temperature in the morning. However, the decreasing temperature made it decrease in the afternoon. The results revealed that respiration of wetland plants was influenced by temperature.

Root oxygen release rates were calculated by mass balance method and plotted in Fig. 5. The mass balance has considered the following components: respiratory oxygen consumption of the roots; oxygen required for degradation of the organic matters; oxygen required for nitrification; and the amount of dissolved oxygen in the influent, effluent and substrate water. The daily changes of PAR were also examined. Root oxygen release rate is ranged from 20.3 to 58.3$gO_2/m^2.d$. The peak value of oxygen release rate (54.0-58.3$gO_2/m^2.d$) was observed during the daytime at 15:00 hrs while the maximum light intensity was observed at 13:00 hrs. Obviously, the peak of root oxygen release appeared after the peak of light intensity. Light intensity data have been demonstrated to predict the root oxygen release rate via the Gaussian function [16], which is in the form of follows:

$$V_O = ae^{-\frac{(t-t_{Omax})^2}{c^2}} \quad (5)$$

Where t is time (4:00am~20:00pm); a (in Gaussian function) is the maximum

value of oxygen release rate in a whole day; t_{Omax} is the location of the symmetry axis in Gaussian function; *c* expresses the gradient of Gaussian function. Decrease in *c* is relative to steep Gaussian function while increase in *c* is relative to gentle Gaussian function.

Light intensity data during daytime (4:00am-20:00pm) also follow Gaussian function (Fig. 5(b)), it can be described as:

$$PAR = be^{-\frac{(t-t_{Lmax})^2}{d^2}} \quad (6)$$

Where *PAR* is the photosynthetically active radiation, μmol·m^{-2} s^{-1}; *t* is time; *b* is the peak value of *PAR* in a whole day; and *d* is the gradient of unimodal.

The Gaussian parameters calculated by the light intensity data of the current study are: b_1=1094, d_1=3.477; b_2=1465, d_2=3.47; a_1=249.00, c_1=3.59; a_2=467.85, c_2=3.58. Then, the root oxygen release rate was predicted and jointly illustrated in Fig. 5. The predicted values were slightly lower than the mass balance results. Oxygen release is increased gradually with increasing light intensity in the morning. However, a decrease in the oxygen release rates occured following the decreased light intensity in the afternoon. It appeared a large difference of root oxygen release for mass balance results and predicted values at night. These variations indicate a significant time dependent interval for oxygen release by plants during day and night.

4. Discussion

Based on the oxygen source and sink in the CW mesocosm, a mass balance for oxygen was calculated. Regarding the consideration of the oxygen sources, it should be

pointed out that the amount of reaeration in subsurface CW was ignored in the mass balance calculations because the levels were generally low. As described by Horn and Smucker [17], oxygen diffusion is limited by partial or complete water saturation. Accordingly, the anoxic sites may be developed. The influent DO was $0.9gO_2/m^2.d$ in average (Fig. 3). With regard to the oxygen sink, the average amount of oxygen required for degradation of the organic and nitrogen-nitrification was $13.8gO_2/m^2.d$ and $3.4gO_2/m^2.d$, respectively (Fig. 2). The dissolved oxygen in the effluent and substrate water was respectively $0gO_2/m^2.d$ and $0.2gO_2/m^2.d$ (Fig. 3). The respiratory oxygen consumption of the roots and rhizomes was almost $21.9gO_2/m^2.d$ (Fig. 4). Finally, the oxygen released by root systems of wetland plant could then be obtained, which was $38.4gO_2/m^2.d$. Such the amount of oxygen release rate could be termed as "net specific oxygen release rate".

The DO budget showed that only 35.0% and 8.7% of the oxygen released by roots were used in the degradation of organic matters and nitrogen-nitrification through heterotrophy bacterial. 55.8% was used for roots respiration, this is because the oxygen released from one part of the root system was reabsorbed by another. The respiratory oxygen consumption of the roots could almost balance the oxygen release, leaving little to be released to the surrounding medium. Such the amount of oxygen release rate could be termed as "dischargeable oxygen release rate". Similar responses/patterns have been reported in Eldridge and Morse [18], who showed that the rate of oxygen consumption exceeds its rate of input from photosynthetic generation.

It is interesting that the results of DO budget could be used to explain the debate

about the quantity of oxygen released by root systems of wetland plant. It is noted from Bezbaruah and Zhang [19] that plants did not release enough oxygen to meet the total oxygen demand of bulk wastewater. The maximum oxygen release rate of only 11.0-12.5mgO_2/kgwet.h (0.34-0.39 μmol O_2/gDW_{root}.h) was reported by Soda et al. [20]. These results can be treated as the "dischargeable oxygen release rate". Since the root oxygen release rate was monitored in oxygen-scavenging solution (or wastewater) using DO microelectrode. The oxygen released from roots was reabsorbed due to the respiration. Contrarily, the obviously higher oxygen release rate of 148-798μmolO_2/gDW_{root}h for *M. spicatum* and *P. crispus* was reported by Laskov et al. [21]. These results can be treated as "net specific oxygen release rate". It should be noted that the high result was obtained because the titanium (III) redox buffer was adopted to determine the oxygen release. The titanium (III) redox buffer could scavenge the oxygen as it was released, preventing it from being reabsorbed by the respiring root tissue. When titanium citrate was used in a closed chamber, the rate of root oxygen release was >80 times higher than in the nutrient solutions [22].

Root oxygen release rates obtained from two methods are depended largely on the light intensity, which has exhibited a diurnal periodic variation. The daily changes of oxygen release and light intensity followed unimodal distribution [16]. The diurnal redox fluctuation as a result of photosynthetic activity of the plant may be a better explanation for this phenomenon. Oxygen is produced through photosynthesis [23] and is transferred from the leaves to the roots of plants via pressurized convective through flow and molecular diffusion within the lacunal system of intercellular airspaces. The

pressure differential is generated by gradients in temperature and water vapour pressure between the internal gas spaces and the surrounding atmosphere [24]. The oxygen concentration is higher within the roots than the rhizosphere. Thus, oxygen is released from roots, where reciprocating concentration gradients of oxygen. Presently, there are several methods of the oxygen transfer measurement, such as microelectrode measurement method [19, 20], oxygen-depleted solution and titanium (III) citrate buffer [10, 22, 25] etc. However, the oxygen release from root systems in field could be changed. Since the special rhizosphere environment in CW could affect the oxygen release. Firstly, the moved wastewater in CW stimulated root oxygen release. The oxygen released by root systems of plants could be easily transferred far away from rhizosphere with increasing flow rate. Secondly, the root systems were growing in the void of substrate. It affected the root morphology. Thirdly, the variations of dissolved oxygen concentration in substrate water could affect the oxygen release. Therefore, the mass balance method in this study could ideally describe the variations of oxygen release in situ.

Another important fact for calculation the oxygen transfer into the rhizosphere is the number of plants per unit of the surface and the mass of the roots. The number of plants was reported about 3 plants/m^2 [26]. Dry weight of plant roots was averaged as 1.19g/plants. In this study, root oxygen release rate calculated by mass balance method were slightly higher than the predicted values since the degradation processes of partial organic and nitrogen compounds were mainly anaerobic (especially at night). However, it was considered as aerobic process for the mass balance method.

5. Conclusions

Root oxygen release rate calculated by mass balance method is ranged from 20.3 to 58.3gO_2/m^2.d. The peak value of oxygen release rate (54.0-58.3gO_2/m^2.d) was observed during the daytime at 15:00 hrs while the maximum light intensity was observed at 13:00 hrs. The results showed that the rates of root oxygen release depend largely on the light intensity, which was exhibited a diurnal periodic variation. The daily changes of oxygen release and light intensity followed unimodal distribution. Only minority (about 35.0% plus 8.7%) of the oxygen released by roots of wetland plant were used in the degradation of organic matters and nitrogen-nitrification. However, majority (55.8%) was used for roots respiration. Based on DO budget, root oxygen release rate could be described by two fractions, including “net specific oxygen release rate” and “dischargeable oxygen release rate”. This could help to explain the debate about the quantity of oxygen released by root systems of wetland plant.

Acknowledgements

This study work was financially supported by the National Natural Science Foundation of China (50979028) and the Ministry of Water Resources of China (200801065).

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379 Fig. 1. Schematic description of the vertical subsurface flow CW system

380 Fig. 2. Diurnal fluctuation of oxygen consumption rate for organic matter degradation and

381 nitrification

382 Fig. 3. Diurnal fluctuation of DO concentration in substrate water, inflow and outflow

383 Fig. 4. Diurnal fluctuation of root respiration rate

384 Fig. 5. Diurnal fluctuation of root oxygen release rate and PAR

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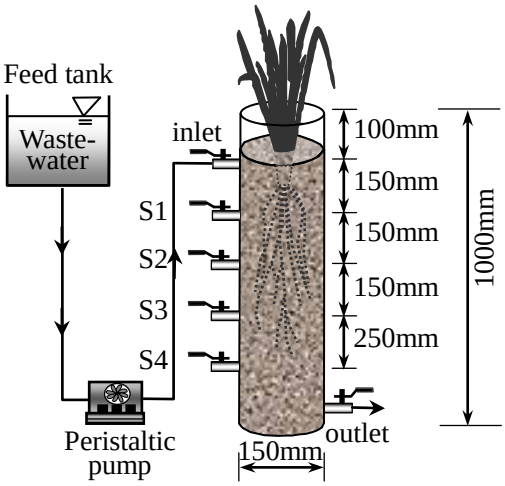

Fig. 1. Schematic description of the vertical subsurface flow CW system

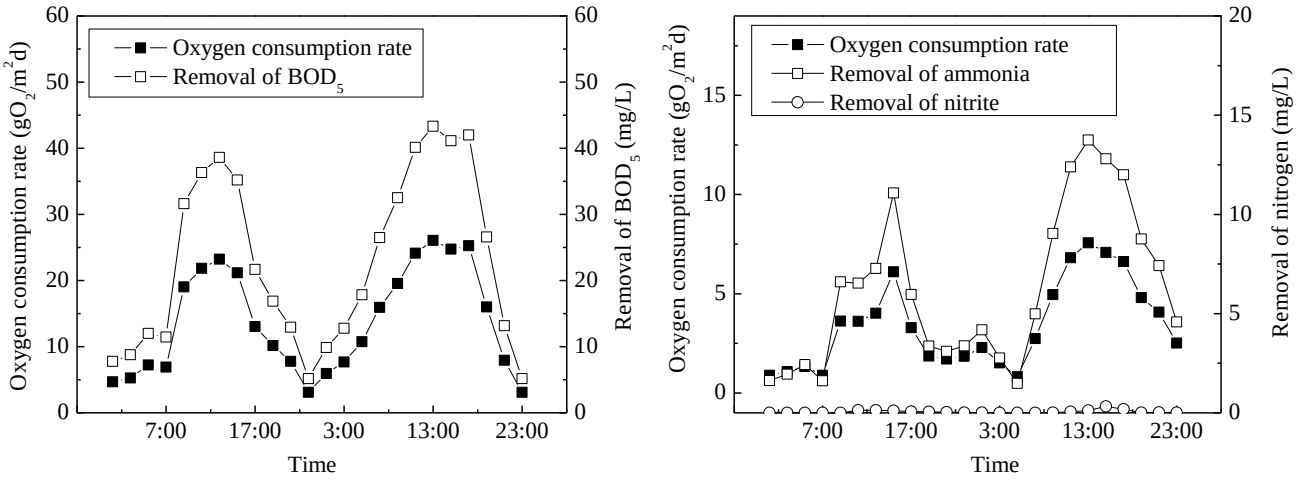

Fig. 2. Diurnal fluctuation of oxygen consumption rate

for organic matter degradation and nitrification

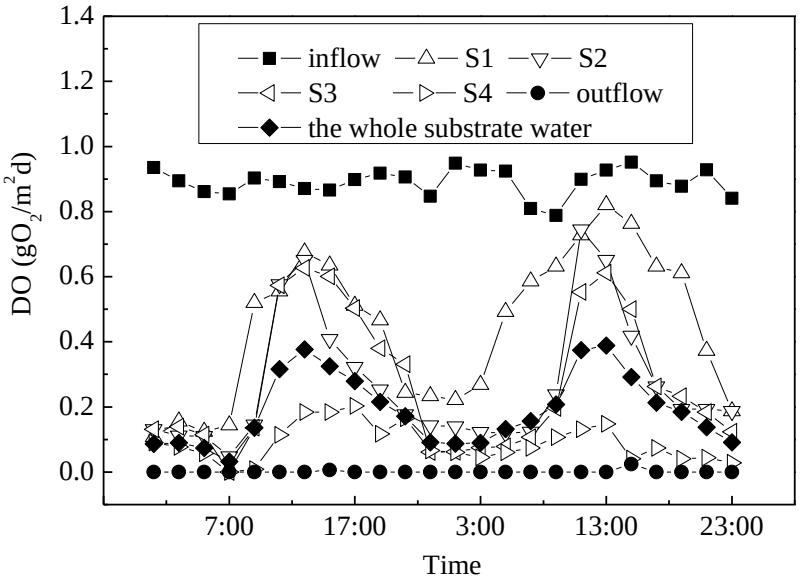

Fig. 3. Diurnal fluctuation of DO concentration in substrate water, inflow and outflow

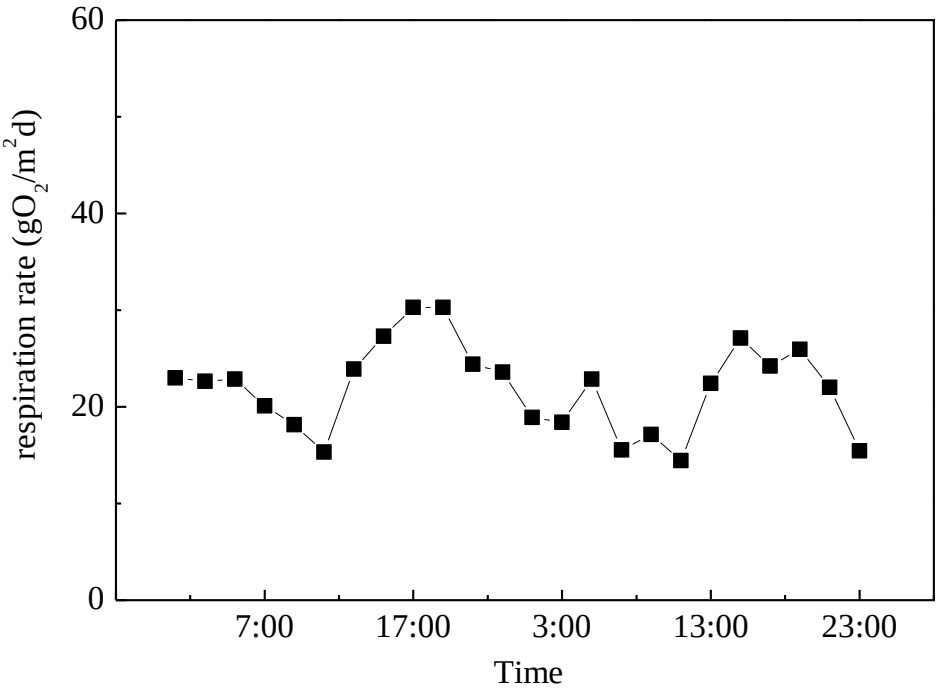

Fig. 4. Diurnal fluctuation of root respiration rate

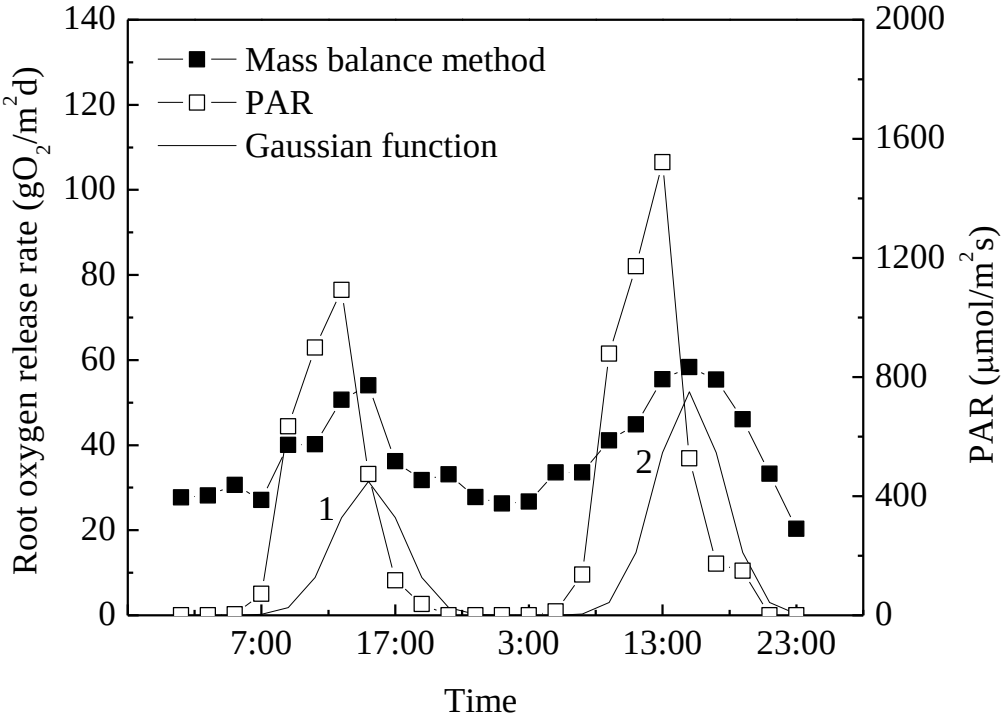

Fig. 5. Diurnal fluctuation of root oxygen release rate and PAR