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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.

24   experimentally evaluated the amount of root oxygen release rate using mass balance

25   method. The mass balance calculation is based on the following components:

26   respiratory oxygen consumption of the roots; oxygen required for degradation of the

27   organic matters; oxygen required for nitrification; and the amount of dissolved oxygen

28   in the influent, effluent and substrate water. Experimental results have demonstrated

29   that the root oxygen release rate was ranged from 20.3 to $58.3 gO_2/m^2.d$ with average

30   value of $38.4 gO_2/m^2.d$, which was affected dramatically by light intensity. Only 35%

31   and 9% of the oxygen released by roots were used in the degradation of organic

32   matters and nitrogen-nitrification, respectively, while 56% was used for roots

33   respiration with little to be released to the surrounding medium. The results also

34   showed that diurnal fluctuation of oxygen release and light intensity followed

35   unimodal distribution. Meanwhile, a better understanding of the DO (dissolved oxygen)

36   budget was proposed. Root oxygen release rate could be described by two fractions.

37   One is "net specific oxygen release rate" and the other is "dischargeable oxygen

38   release rate".

41

1. Introduction

43   The global use of constructed wetlands (CW) as an eco-friendly, low maintenance,

44   low-cost, and efficient alternative to conventional wastewater treatment has been

45   growing over the last decades [1,2]. Pollutants are reduced from wastewater in the CW

46 by a variety of physiochemical and biological processes. Available oxygen in CW is an

47 important factor in degradation of organic matter and transformation of

48 ammonium-nitrogen, both of which are oxygen limiting processes. When hypoxia

49 (dissolved oxygen <2mg/L) occurs, a wide range of negative effects on aquatic

50 ecosystems and organisms could be caused [3].

51   Coupled nitrification-denitrification is considered as the main N sink in CW [4].

52 Nitrification is an aerobic chemoautotrophic process while denitrification is an

53 anaerobic heterotrophic microbial process. The nitrification step represents the main

54 limiting factor for N removal in CW because of low oxygen availability in most cases

55 [5,6]. Low oxygen content results in low aerobic organic matter decomposition [6,7].

56 In order to enhance N removal efficiency, oxygen must be provided to the nitrifying

57 microbes through oxygenation of the wetland matrix via several means. Plants provide

58 oxygen to the rhizosphere via passive or active oxygen transport through their stems

59 from the atmosphere to the roots resulting in higher N removal rates in planted CW [8].

60   Recently, the interest in the main term of oxygen is increasing. Many studies have

61 been conducted to illustrate the mechanisms responsible for mass balance of DO

62 (dissolved oxygen) in estuarine and coastal areas worldwide [9]. However, there is

63 little detailed information about the oxygen mass balance in CW. Furthermore, only

64 few attempts have yet been made to evaluate the diurnal changes of oxygen

65 consumption during degradation of organic matter or nitrogen oxidation. Accordingly,

66 little was known about the diurnal variation of oxygen release by the roots of wetland

67 plant. The role of plants for oxygen offering remains unclear. Therefore, the objective

68 of the present study was to provide a better understanding of the DO budget and the

69 daily variation of the root oxygen release rates, which were explored based on mass

70 balance method.

71

72 **2. Materials and methods**

73 *2.1. Experimental mesocosm*

74 The model wetland used in this study was a sub-surface vertical flow CW.

75 Mesocosm was prepared from perspex column of 150mm in diameter and 1000mm in

76 height. The mesocosm was filled with 2-5mm sized scoria as the substrate in a depth of

77 900 mm, giving an average porosity value of 0.46. Four sampling ports (S1, S2, S3,

78 S4), inlets and outlets at different heights of the mesocosm (model CW) were setup

79 (Fig. 1). Young *Acorus calamus* Linns collected from a natural wetland located in

80 Xuanwu Lake, Nanjing, China, were planted in the wetland mesocosm. It was exposed

81 to the open air with natural light outside the laboratory building.

82 Ammonium chloride, potassium dihydrogen phosphate and glucose were used as

83 the sources of ammonium and organic matters. They were mixed with tap water to

84 prepare the artificial wastewater [10]. The characteristics of the wastewater were as

85 follows: TN 15.1mg/L (ranged between 12.0-18.7mg/L), NH_4^+-N 13.6 (ranged

86 between 12.1-15.2 mg/L), NO_3^--N 1.7 mg/L (ranged between 1.5-1.8 mg/L) and

87 NO_2^--N 0.006 mg/L (ranged between 0-0.011 mg/L). The wastewater was pumped into

88 a holding tank, from where the mesocosm was supplied. Hydraulic load rate (HLR)

89 was $0.16m^3/m^2$ d, and the hydraulic retention time was about 2.43d. The model CW

90 was operated for one year. When the mesocosm reached its steady state after the

91 start-up period, Samples of influent and effluent were collected from the six sampling

92 points along the mesocosm every two hours. The samples were then analysed for DO,

93 BOD_5, total nitrogen, ammoniacal-nitrogen, nitrite and nitrate according to the

94 Standard Methods for Examination of Water and Wastewater [11]. Light intensity was

95 measured every two hours using a luminometer (MODEL ZDS-10F-2D). The unit of

96 light intensity is lux. In this paper, light intensity was described as photosynthetically

97 active radiation (PAR). One lux is 0.019 $\mu mol \cdot m^{-2} \ s^{-1}$ [12].

98

99 *2.2. Respiration of wetland plants*

100 To prepare the examination, the surface of substrate (scoria) was covered with

101 vaseline to prevent re-aeration from the atmosphere. The plants were covered with a

102 black plastic bag to prevent photosynthesis. These were to make sure that the

103 respiration was mainly taking place in the substrate. For the purpose of examining the

104 respiration of wetland plants, the oxygen in the blank influent (without pollutants) of

105 the wetland mesocosm was carefully pre-monitored. Such the oxygen was then

106 absorbed by the root systems of plant in the process of respiration. Two hours later, the

107 oxygen in effluent was examined. The difference of oxygen (in mass) can be used to

108 calculate respiration rate.

109 The difficulty of the examination is the disturbance of oxygen from the air. The

110 details of the procedure were as follow: Firstly, the volume of the blank influent was

111 carefully controlled and the DO concentration was pre-monitored. Secondly, before the

112 blank influent was introduced to the mesocosm, the O_2 in the pore space of the

113 substrate was replaced by N_2. The influent was then put into the mesocosm suddenly.

114 Two hours later, the mesocosm was carefully drained using a vacuum-pump. At the

115 same time, N_2 was injected into the mesocosm to prevent re-aeration. Finally, the

116 volume and DO concentration of the effluent of the wetland mesocosm was examined,

117 this allows to calculating the O_2 mass.

118

119 *2.3. Statistical analyses*

120

121 *2.3.1. Oxygen consumption rate calculations.*

122 The reasonable way to calculate the DO consumed by bacteria is via the

123 decomposition of the organic matters [13]. Organic pollutants in wastewater are

124 adsorbed on microbial cell surface which inhabits on the substrate surface. Pollutants

125 are metabolized by microbes under catalysis of various enzymes. Oxygenolysis of

126 partial organic matter by microbes is called catabolic metabolism. The other organic

127 pollutants are taken in by microbes, and converted to new cells, this process is called

128 anabolism. Based on these two metabolic processes, the oxygen demand of reducing

129 organic matter ($O_{organic}$, mg/L) was analyzed theoretically as follows [13, 14]:

130 $$O_{organic} = a^{'}BOD_r + b^{'}P \tag{1}$$

131 Where the first part is the oxygen consumed in the process of catabolic

132 metabolism. The second part is the oxygen consumed in the process of anabolism. a' is

133 oxygen demand for the complete degradation of 1kg BOD_5; BOD_r is the removal of

134   BOD_5 in substrate, mg/L; b' is the oxygen demand of active biofilm per unit weight; P

135   is the quantity of active biofilm which adsorbs to every 1 m^3 substrate (kg/m^3). Oxygen

136   consumption rate ($gO_2/m^2 d$) could be calculated as multiplying the oxygen demand by

137   hydraulic load rate ($0.16 m^3/m^2$ d). It is noted that Ye [13] reported that $b'P$ value was

138   to be $0-0.00042 kg/m^3$ at different depths. Therefore, $b'P$ could be ignored. Here, the

139   value of a' is 3.76 ($kgO_2/kgBOD_5$) since 3.53kg glucose is corresponding to $1kgBOD_5$

140   while 1kg glucose is corresponding to 1.07kg oxygen.

141   Oxygen consumed by nitrification was calculated based on Eq. (2) and Eq. (3).

142   Oxidizing 1g of NH_4^+-N and NO_2^--N requires 3.43g and 1.14g of oxygen, respectively.

143
$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \tag{2}$$

144
$$2NO_2^- + O_2 \rightarrow 2NO_3^- \tag{3}$$

145
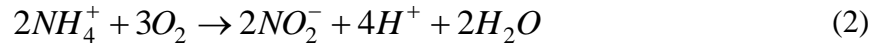
146   *2.3.2. Oxygen mass balance calculations.*

147   In CW mesocosm, the root oxygen release could be assessed with mass balance

148   method [15]. The main sources of oxygen in the substrate water include the inflow

149   carrying, aeration and oxygen releasing from roots of plant. Output of oxygen includes

150   outflow carrying, nitrification consumption and organic mater consumption. Mass

151   balance equation of oxygen is given below:

152
$$O_{plant} = (O_{out} + O_{organic} + O_{nitrification} + O_{substract} + O_{res}) - O_{in} \tag{4}$$

153   Where O_{plant} is root oxygen release, $gO_2 m^{-2} d^{-1}$; O_{out} is outflow carrying, $gO_2 m^{-2} d^{-1}$;

154    $O_{organic}$ is organic matter consumption, $gO_2 m^{-2} d^{-1}$; $O_{nitrification}$ is nitrification

155   consumption, $gO_2 m^{-2} d^{-1}$; $O_{substract}$ is DO in substrate water, $gO_2 m^{-2} d^{-1}$; 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 58.3gO_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. 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:

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

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

200   value of oxygen release rate in a whole day; t_{Omax} is the location of the symmetry axis

201   in Gaussian function; c expresses the gradient of Gaussian function. Decrease in c is

202   relative to steep Gaussian function while increase in c is relative to gentle Gaussian

203   function.

204   Light intensity data during daytime (4:00am-20:00pm) also follow Gaussian

205   function (Fig. 5(b)), it can be described as:

206
$$PAR = be^{-\frac{(t-t_{Lmax})^2}{d^2}} \tag{6}$$

207   Where PAR is the photosynthetically active radiation, $\mu mol \cdot m^{-2} \ s^{-1}$; t is time; b is

208   the peak value of PAR in a whole day; and d is the gradient of unimodal.

209   The Gaussian parameters calculated by the light intensity data of the current study

210   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.

211   Then, the root oxygen release rate was predicted and jointly illustrated in Fig. 5. The

212   predicted values were slightly lower than the mass balance results. Oxygen release is

213   increased gradually with increasing light intensity in the morning. However, a decrease

214   in the oxygen release rates occured following the decreased light intensity in the

215   afternoon. It appeared a large difference of root oxygen release for mass balance results

216   and predicted values at night. These variations indicate a significant time dependent

217   interval for oxygen release by plants during day and night.

218

219   **4. Discussion**

220   Based on the oxygen source and sink in the CW mesocosm, a mass balance for

221   oxygen was calculated. Regarding the consideration of the oxygen sources, it should be

222   pointed out that the amount of reaeration in subsurface CW was ignored in the mass

223   balance calculations because the levels were generally low. As described by Horn and

224   Smucker [17], oxygen diffusion is limited by partial or complete water saturation.

225   Accordingly, the anoxic sites may be developed. The influent DO was $0.9gO_2/m^2.d$ in

226   average (Fig. 3). With regard to the oxygen sink, the average amount of oxygen

227   required for degradation of the organic and nitrogen-nitrification was $13.8gO_2/m^2.d$

228   and $3.4gO_2/m^2.d$, respectively (Fig. 2). The dissolved oxygen in the effluent and

229   substrate water was respectively $0gO_2/m^2.d$ and $0.2gO_2/m^2.d$ (Fig. 3). The respiratory

230   oxygen consumption of the roots and rhizomes was almost $21.9gO_2/m^2.d$ (Fig. 4).

231   Finally, the oxygen released by root systems of wetland plant could then be obtained,

232   which was $38.4gO_2/m^2.d$. Such the amount of oxygen release rate could be termed as

233   "net specific oxygen release rate".

234   The DO budget showed that only 35.0% and 8.7% of the oxygen released by roots

235   were used in the degradation of organic matters and nitrogen-nitrification through

236   heterotrophy bacterial. 55.8% was used for roots respiration, this is because the oxygen

237   released from one part of the root system was reabsorbed by another. The respiratory

238   oxygen consumption of the roots could almost balance the oxygen release, leaving

239   little to be released to the surrounding medium. Such the amount of oxygen release rate

240   could be termed as "dischargeable oxygen release rate". Similar responses/patterns

241   have been reported in Eldridge and Morse [18], who showed that the rate of oxygen

242   consumption exceeds its rate of input from photosynthetic generation.

243   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

266 pressure differential is generated by gradients in temperature and water vapour

267 pressure between the internal gas spaces and the surrounding atmosphere [24]. The

268 oxygen concentration is higher within the roots than the rhizosphere. Thus, oxygen is

269 released from roots, where reciprocating concentration gradients of oxygen. Presently,

270 there are several methods of the oxygen transfer measurement, such as microelectrode

271 measurement method [19, 20], oxygen-depleted solution and titanium (III) citrate

272 buffer [10, 22, 25] etc. However, the oxygen release from root systems in field could

273 be changed. Since the special rhizosphere environment in CW could affect the oxygen

274 release. Firstly, the moved wastewater in CW stimulated root oxygen release. The

275 oxygen released by root systems of plants could be easily transferred far away from

276 rhizosphere with increasing flow rate. Secondly, the root systems were growing in the

277 void of substrate. It affected the root morphology. Thirdly, the variations of dissolved

278 oxygen concentration in substrate water could affect the oxygen release. Therefore, the

279 mass balance method in this study could ideally describe the variations of oxygen

280 release in situ.

281 Another important fact for calculation the oxygen transfer into the rhizosphere is

282 the number of plants per unit of the surface and the mass of the roots. The number of

283 plants was reported about 3 plants/m^2 [26]. Dry weight of plant roots was averaged as

284 1.19g/plants. In this study, root oxygen release rate calculated by mass balance method

285 were slightly higher than the predicted values since the degradation processes of partial

286 organic and nitrogen compounds were mainly anaerobic (especially at night). However,

287 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.

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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

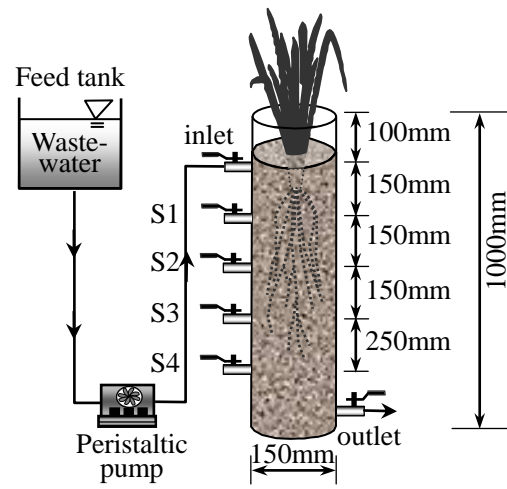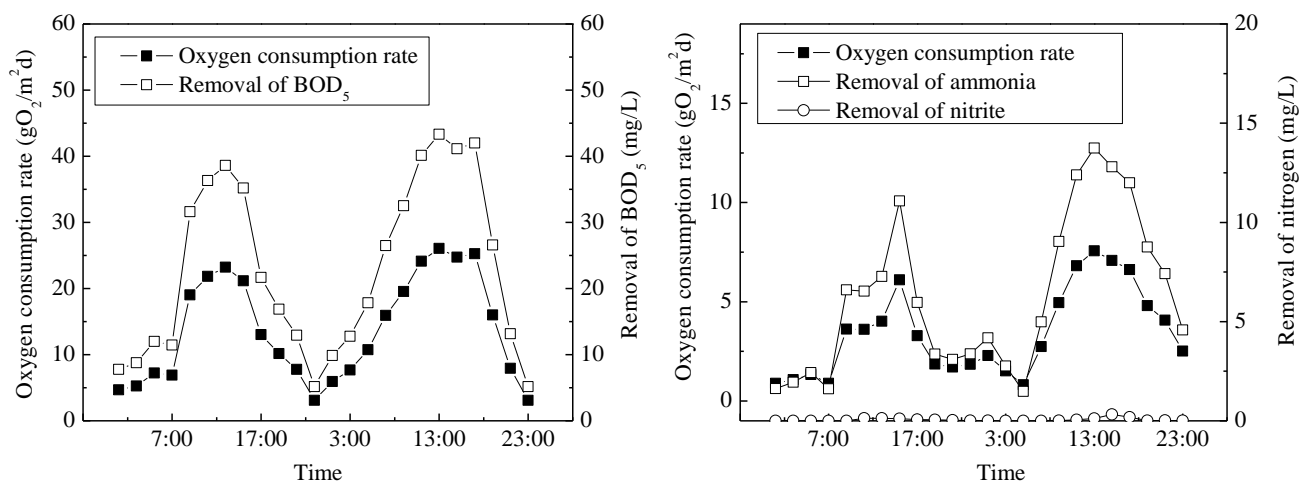384   Fig. 5. Diurnal fluctuation of root oxygen release rate and PAR

385

386

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

Fig. 2. Diurnal fluctuation of oxygen consumption rate

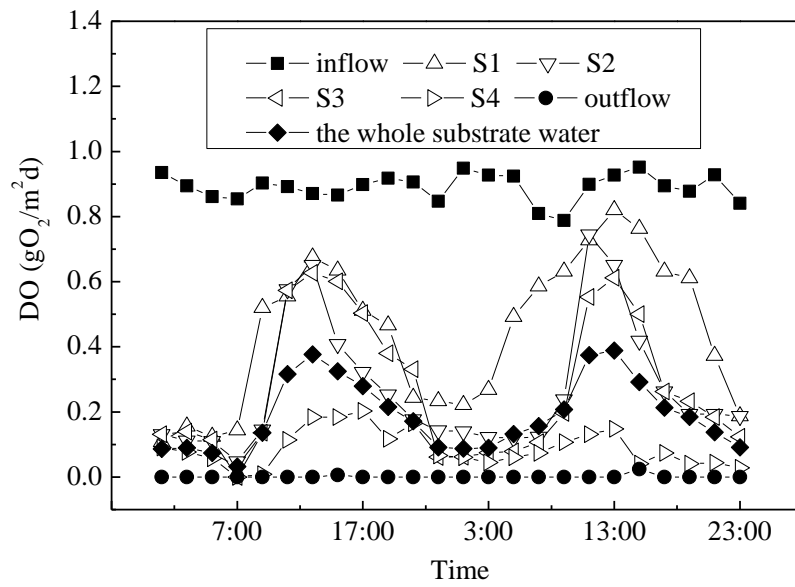for organic matter degradation and nitrification

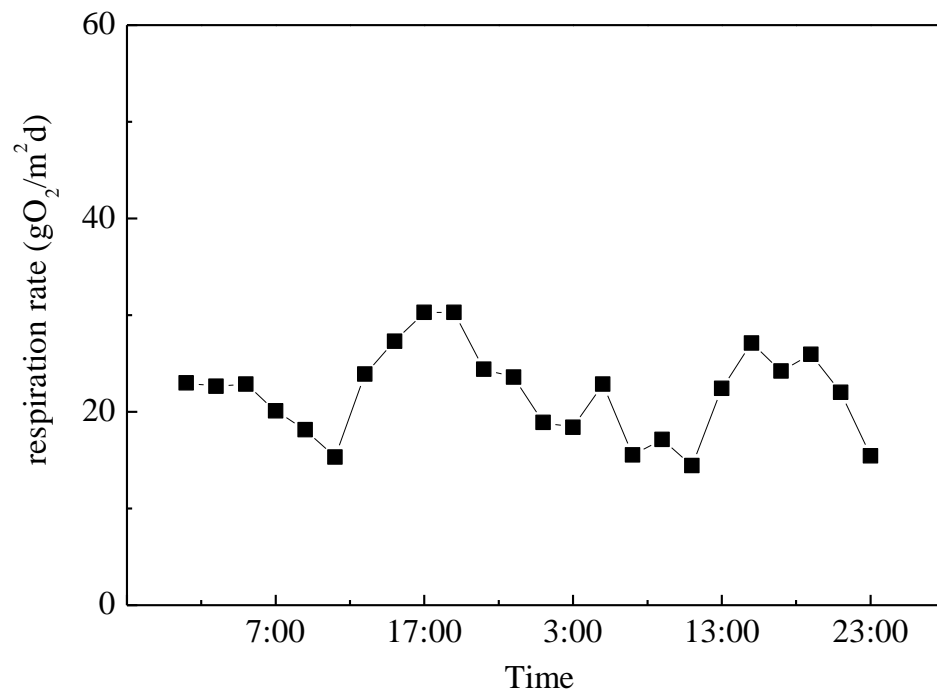Fig. 3. Diurnal fluctuation of DO concentration in substrate water, inflow and outflow

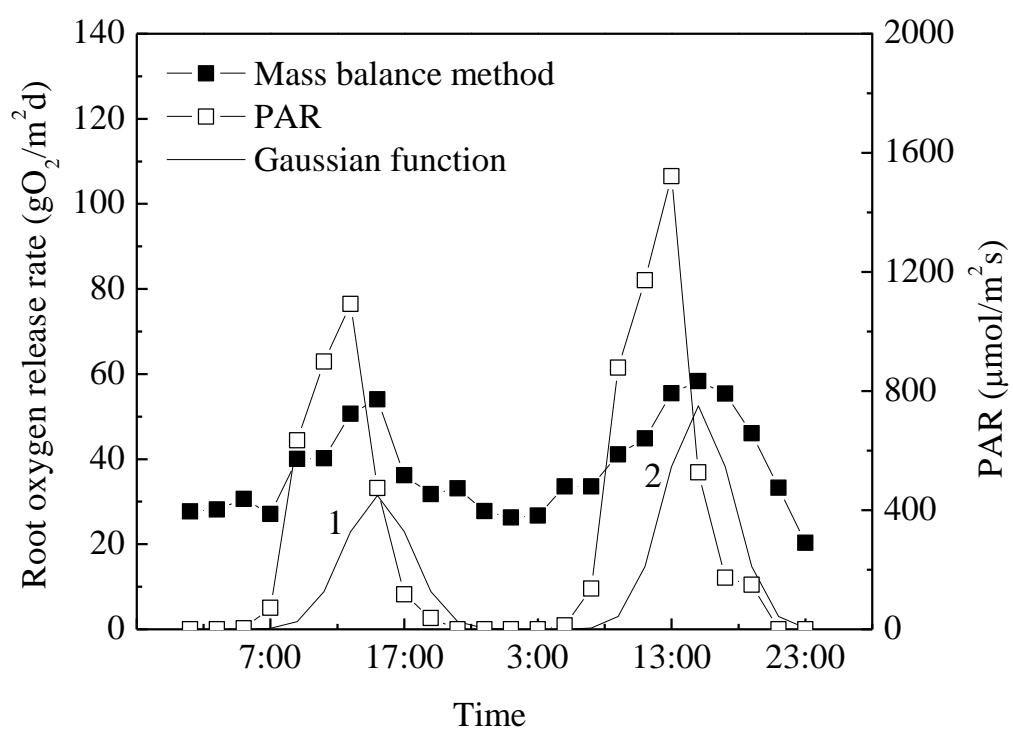Fig. 4. Diurnal fluctuation of root respiration rate

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