Effect of plant harvesting on the performance of constructed wetlands during winter: radial oxygen loss and microbial characteristics

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Abstract

The aboveground tissue of plants is important for providing roots with constant photosynthetic resources. However, the aboveground biomass is usually harvested before winter to maintain the permanent removal of nutrients. In this work, the effects of harvest on plants' involvement in oxygen input as well as in microbial abundance and activity were investigated in detail. Three series of constructed wetlands with integrated plants ("unharvested"), harvested plants ("harvested"), and fully cleared plants ("cleared") were set up. Better performance was found in the unharvested units, with the radial oxygen loss (ROL) rates ranging from 0.05 to 0.59 μmol O_2/h/plant, followed by the harvested units that had relatively lower ROL rates (0.01 to 0.52 μmol O_2/h/plant). The cleared units had the lowest removal efficiency, which had no rhizome resources from the plants. The microbial population and activity were highest in the unharvested units, followed by the harvested and cleared units. Results showed that bacterial abundances and enhanced microbial activity were ten times higher on root surfaces compared with sands. These results indicate that late autumn harvesting of the aboveground biomass exhibited negative effects on plant ROL as well as on the microbial population and activity during the following winter.

Keywords

Harvesting, Constructed wetlands, Radial oxygen loss, Microbial population, Microbial activity

Introduction

Given their inexpensive cost and low power consumption, constructed wetlands (CWs) are increasingly used for wastewater treatment (Vymazal 2010). Plants, as the primary components of CWs, possess an important role in pollutant removal because of their uptake, storage, and release processes (Dickopp et al. 2011). However, the removal efficiency of CWs is challenged during winter with the reduced biological removal pathways and nutrients released because of plant decay (Kadlec and Wallace 2008, Wang et al. 2012a). The aboveground biomass of plants in CWs is usually harvested before winter to maintain permanent pollutant removal (Granéli et al. 1992; Jinadasa et al. 2008).

Aboveground tissue is important for nutrient storage and removal processes in wetlands (Toet et al. 2005). Plant shoots in the water column can decrease the current velocity of the through-flowing water and are critical for the delivery of oxygen and organic compounds from aboveground organs to belowground parts. Thus, aboveground biomass harvesting may affect the plant itself and the removal processes (Asaeda and Karunaratne 2000; Asaeda et al. 2006). Plants react promptly to disturbances to their internal equilibrium (Valkama et al. 2008). Therefore, we speculated that the influences of plant physiological changes caused by harvesting on the following winter removal efficiency could not be neglected.

Plants' involvement in oxygen input is important for the activity and types of metabolism performed by microorganisms (Stottmeister et al. 2003). Some of the photosynthetic oxygen produced in the leaves of emergent plants and the atmospheric oxygen entering the shoots by diffusion via the stomata are transported to underground tissues through aerenchymal tissues (Armstrong et al. 1992; Armstrong 1978). Some of the oxygen is consumed during root respiration or transported back into the atmosphere; the remaining oxygen is leaked from the root region to the rhizosphere in a process called radial oxygen loss (ROL) (Brix and Schierup 1990; Tanaka et al. 2007). Thus, ROL causes the oxygen concentration to be much higher in the rhizosphere than in the surrounding soil (Van Bodegom et al. 2001). Oxygen leakage has a significant impact on important mechanisms of wastewater treatment in CWs, including its influence on the redox potential (Białowiec et al. 2012) and its enhancement of microbial activity (Ueckert et al. 1990).

Tanaka et al. (2007) found that plants with open dead culms have higher ROL rates than those with sealed dead culms. However, the higher oxygen transport through open dead culms is more pronounced under windy conditions (Armstrong et al. 1992). Therefore, harvesting has a potential influence on the oxygen leakage from plants. The ROL rates in the present study were detected during harvesting operations. Microbial abundance and activity were also investigated to gain insights into the environmental impact of harvesting.

Three series of wetlands with integrated plants, harvested plants, and fully cleared plants were set up. We directly quantified the ROL rates and analyzed the microbial population and activity using quantitative polymerase chain reaction (qPCR) assays based on 16S rRNA and the functional ammonia-monooxygenase (*amo*A) gene. This study aimed to provide a

theoretical understanding of the effects of harvesting on wetland performance in the subsequent winter.

Materials and methods

Site description and operation

Experiments were performed in nine independent microcosm wetland systems located in Shang Dong Normal University in Jinan, China (36° 40′ 36″ N, 117° 3′ 42″ E), which had a warm-temperature monsoonal climate. The microcosm wetland systems were set up using a subsurface flow design for treating domestic wastewater on March 2, 2012. The treatment cells were polyvinyl chloride columns (48 cm deep and 30 cm in diameter) filled with 15 mm gravel and washed sand (particle size <2 mm) to depths of 5 and 35 cm, respectively (Fig. 1). Each cell was planted with *Phragmites australis*, which was transplanted from Nansi Lake. The plant density was 17 rhizomes per cell. The microcosms were fed synthetic wastewater to simulate the post-primary (B) domestic wastewater effluent (Taylor et al. 2011). The influent with 14.45 ± 0.24 mg/L ammonia nitrogen (NH_4^+-N) and 59.38 ± 4.08 mg/L chemical oxygen demand (COD) was pumped into the cells to keep the water level below the sand surfaces. Each wetland was batch-operated for 7 days. Plants were grown for 8 months before harvesting. By the end of autumn (November 24, 2012), plant shoots were harvested in three cells (“harvested” group); then, both plant shoots and roots were harvested in another three cells (“cleared” group), and the remaining cells with unharvested plants were used as the controls (“unharvested” group). The day of the harvest was deemed the first day of this study.

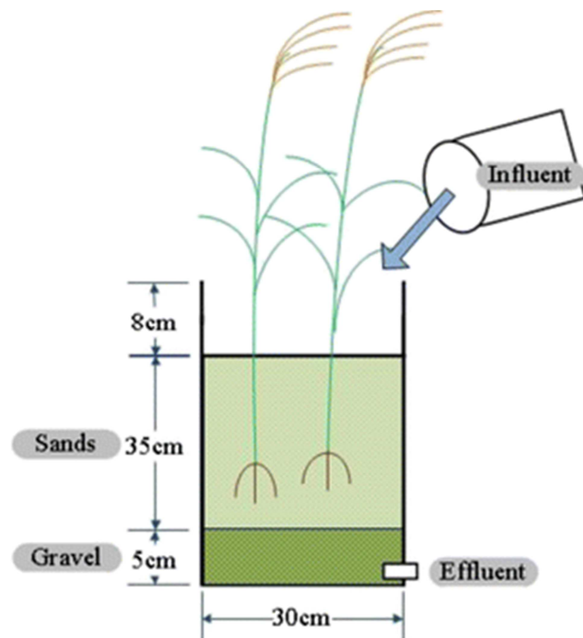

Fig. 1 Profile of the pilot-scale constructed wetland unit

Sampling

The dissolved oxygen (DO) and temperature (T) of the influent and effluent were measured every 7 days. At the seventh day of each cycle, the influent and effluent water samples were collected and stored in sterile plastic bottles. The water samples were immediately brought to the lab and stored at 4 °C before chemical analysis.

The ROL rates were tested at different temperature stages, namely, 9.43 ± 0.60, 8.48 ± 2.58, 5.56 ± 1.78, and 2.40 ± 1.45 °C, corresponding to the 14th, 21st, 28th, and 42nd day of the experiment. Undamaged plants were removed from the microcosm to quantify the ROL rate. The plants were carefully separated from the debris and cultivated in the same simulated wastewater environment before measurement.

The wetlands were pre-cultured for 8 months before the 42-day experiment. Sand and root samples were obtained for DNA and RNA extraction from each microcosm at the end of the experiment (on the 42nd day). Each microcosm was completely drained before the sand samples were collected from the top layer (5 to 10 cm) in five different plots (Calheiros et al. 2010). Subsequently, the plants were removed and separated from any residues by shaking the plant in a container of sterile water. Roots of approximately 0.5 to 1 mm in diameter near the root tips (Faulwetter et al. 2013) were aseptically excised from the plant. Both the sand and root samples were stored in 5-mL aseptic Eppendorf tubes, immediately placed on ice, and stored at –80 °C before microbial analysis.

Analysis

Chemical analysis

Laboratory analysis was performed on the water samples to determine the COD and ammonium (NH_4^+-N) content. Water temperature and DO were measured using a DO meter (HQ30d 53LEDTM; HACH, USA). All the aforementioned parameters were determined according to standard methods (APHA 2002).

Microbial analysis

DNA and RNA extraction

DNA and RNA were extracted using the respective MOBIO PowerSand™ DNA and RNA Isolation Kits, according to the manufacturer's instructions. DNA and RNA yields were measured with a NanoDrop ND-1000 UV–vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The absorbance ratio (A_{260}/A_{280}) was used to assess protein contamination and their purity. Their integrity was evaluated using formaldehyde denaturing agarose gels. Complementary DNA (cDNA) was generated using the superscript reverse transcriptase (TaKaRa, Japan). The reverse transcription mixture contained 4 μL of 5× PrimeScript® Buffer 2 (for real-time PCR), 1 μL of PrimeScript® RT Enzyme Mix 1, 1 μL of RT Primer Mix, 10 μL of template RNA (≤100 ng), and up to 20 μL of RNase-Free dH_2O (TaKaRa, Japan). The reverse transcription PCR

program is shown in Table 1. The DNA and cDNA products were stored at −20 °C before qPCR analysis.

Table 1 PCR programs used in this study

Application	PCR Program
***amo*A gene cloning**	Initial denaturation for 60 s at 94 °C, followed by 35 cycles of 94 °C for 60 s, 54 °C for 60 s, and 72 °C for 3 min. The program ended with an extension step at 72 °C for 10 min.
16S rRNA gene cloning	Initial denaturation for 10 min at 95 °C, followed by 35 cycles of 95 °C for 10 s, 60 °C for 15 s, and 72 °C for 20 s. The program ended with an extension step at 72 °C for 10 min.
Reverse transcription PCR	Hold at 37 °C for 15 min and 85 °C for 5 s
16S rRNA qPCR	Initial denaturation for 30 s at 95 °C, followed by 40 cycles of: 95 °C for 10 s, 60 °C for 15 s, and 72 °C for 20 s.
***amo*A qPCR**	Initial denaturation for 30 s at 95 °C, followed by 40 cycles of 95 °C for 60 s, 54 °C for 60 s, and 72 °C for 60 s.

qPCR analysis

qPCR was performed using a Roche LC-480 real-time PCR system (CH). A series of 10-fold dilutions of the extract DNA and cDNA were run to assess for possible qPCR inhibition. Inhibition was observed without dilution to test the 16S rRNA copy numbers. Thus, a 100-fold dilution for 16S rRNA was used in the final analysis, and the remaining reactions were performed without dilution (Di et al. 2010). The 16S rRNA and*amo*A genes were quantified using the primers Eub341F/Eub534R (Muyzer et al. 1993) and RottF/RottR (Rotthauwe et al. 1997), respectively, with SYBR® Premix Ex Taq™ (TaKaRa, Japan). The 20 μL of reaction mixture consisted of 10 μL of SYBR® Premix Ex Taq™, 0.4 μL of each of the forward and reverse primers (10 μM), 7.2 μL of nuclease-free water, and 2 μL of template DNA (1 to 10 ng). The qPCR programs are shown in Table 1. The final qPCR data was generated using the Abs Quant/2nd Derivative Max provided with the Roche LC-480 system.

Standard curves were obtained as follows (Di et al. 2010). In brief, the purified PCR products (16S rRNA and*amo*A) obtained from the extracted DNA were cloned into plasmid vectors PMD-18T (TaKaRa, Japan), following the manufacturer's protocol. The plasmids extracted from the correct insert clones of each target gene were sequenced. The edited sequences were compared with known sequences in the GenBank to identify the correct constructed plasmid. The concentration of the plasmid DNA was determined using the Nanodrops ND-1000 UV–vis spectrophotometer, and the target gene copy numbers were directly calculated from the concentration of the plasmid DNA. The 10-fold serial dilutions of the quantified standard plasmids were subjected to a qPCR assay in triplicate to generate external standard curves. The standard curve efficiency was 0.820 for the 16S rRNA gene and 0.915 for the *amo*A gene. The copy number of the standard plasmids ranged from 2.78×10^2 to 2.78×10^7 copies/μL for the 16S rRNA gene and 1.84×10^2 to 1.84×10^7copies/μL for the *amo*A gene.

Radial oxygen loss

After sampling, the plants were carefully separated from the debris and cultivated in the same simulated wastewater. The roots were then placed in a Ti^{3+} citrate solution for 24 h. The flasks were gently shaken initially to even out the color gradients near the roots, and then 5 mL of the solution was removed to measure the color reduction using a spectrophotometer at a wavelength of 527 nm. The concentration of the Ti^{3+} citrate solution was extrapolated according to the standard curve, which was obtained from a serial dilution of Ti^{3+} citrate solutions with known concentrations. The root oxygen release rate was calculated based on the reduction rate of the concentration of the Ti^{3+} citrate solution in the incubation flasks as described by Sasikala et al. (2009).

Statistical analysis

The amount of O_2 (mg/m^3/day) provided by ROL was calculated using the following equation:

$$\text{oxygen concentration}\,(\text{mg/m}^3/\text{day}) = \frac{R \times N \times M}{V} \times 24\ \text{h} \times 10^{-3}$$

where R = ROL rates (μmol O_2/h/plant), N = number of plants per cell, M = molar mass of oxygen (32 g/mol), and V = volume of the cell (m^3). Statistical analysis was performed using SPSS software. Independent samples *t* test was carried out to evaluate the statistical differences of the ROL rates between unharvested and harvested plants.

Results and discussion

Effluent water parameters

Figure 2a, b shows the effluent $NH_4{}^+$-N and COD concentrations for each microcosm. Two conclusions could be deduced. First, the nutrient concentrations in the cleared wetlands were obviously higher than those in the planted wetlands. It means that the presence of the plants enhanced ammonia oxidation and COD removal in winter. Second, the concentrations of $NH_4{}^+$-N and COD in the planted microcosms were much higher in the harvested cells than those in the unharvested cells. This result implies that harvesting operation decreased the $NH_{4\,+}$-N and COD removal during the following winter. Previous studies proved that nitrification limitation and low aerobic organic matter decomposition can be induced by low oxygen availability since ammonia oxidation and COD removal are redox-sensitive processes (Control 2001, Johansson et al. 2004; Wu et al. 2001). Therefore, the effluent oxygen concentrations were detected to explain these phenomena (as shown in Table 2). Our results showed that the DO concentrations in the influents were high during the low winter temperature. Even the cleared microcosms had a higher effluent DO concentration than the planted microcosms, indicating that enough oxygen was supplied to the systems. Previous studies found that microbial bacteria participate in removal processes (Faulwetter et al. 2009).

Thus, we inferred that microbial variation and temperature played a key role in the microcosm. Further discussions are presented in the following section.

Table 2 Effluent temperatures (°C) and dissolved oxygen concentrations (mg/L) of the three experiments (mean ± SD, $n = 3$)

Day	Influent		Unharvested		Harvested		Cleared	
	T	DO	T	DO	T	DO	T	DO
7	11.4 ± 0.5	6.91 ± 0.3	10.1 ± 0.1	1.26 ± 0.5	10.2 ± 0.3	2.14 ± 0.2	10.1 ± 0.1	2.94 ± 0.1
14	8.1 ± 0.2	7.7 ± 0.2	7.1 ± 0.1	0.93 ± 0.2	7.5 ± 0.1	1.24 ± 0.6	7.8 ± 0.3	1.33 ± 0.2
21	6.1 ± 0.3	7.7 ± 0.1	4.7 ± 0.5	1.18 ± 0.1	4.7 ± 0.5	0.91 ± 0.1	4.9 ± 0.1	2.17 ± 0.5
28	6.8 ± 0.1	8.63 ± 0.5	4.1 ± 0.1	1.56 ± 0.8	4.1 ± 0.1	1.23 ± 0.5	4.6 ± 0.3	4.26 ± 0.1
35	5.4 ± 0.1	9.43 ± 0.1	3.3 ± 0.2	1.25 ± 0.1	3.3 ± 0.4	2.28 ± 0.1	3.8 ± 0.2	4.99 ± 0.6
42	0 ± 0.1	10.7 ± 0.5	3.5 ± 0.3	3.26 ± 0.2	3.5 ± 0.2	3.95 ± 0.5	3.9 ± 0.1	6.58 ± 0.5

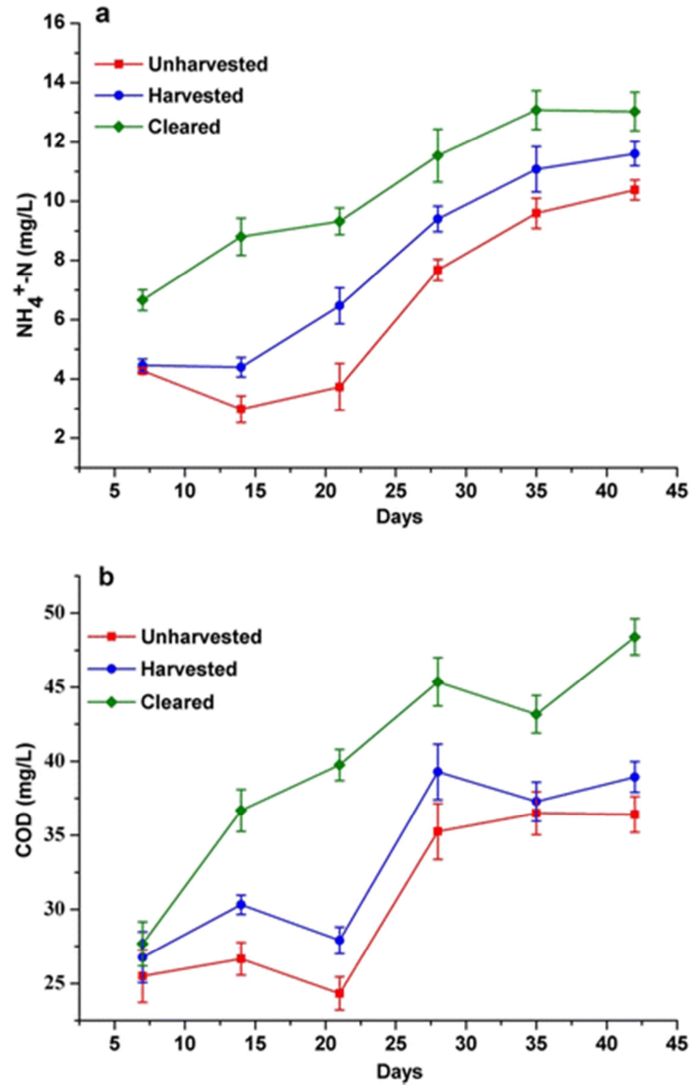

Fig. 2 Comparison of NH_4^+-N (**a**) and COD (**b**) concentrations in effluent water for the three types of experiments at 7-day retention time. *Error bars* represent standard error ($n = 3$)

Microbial abundance and activity

Figure 3 presents the differences of ammonia oxidizing bacteria (AOB) and the total bacteria abundance among the wetlands in sands.

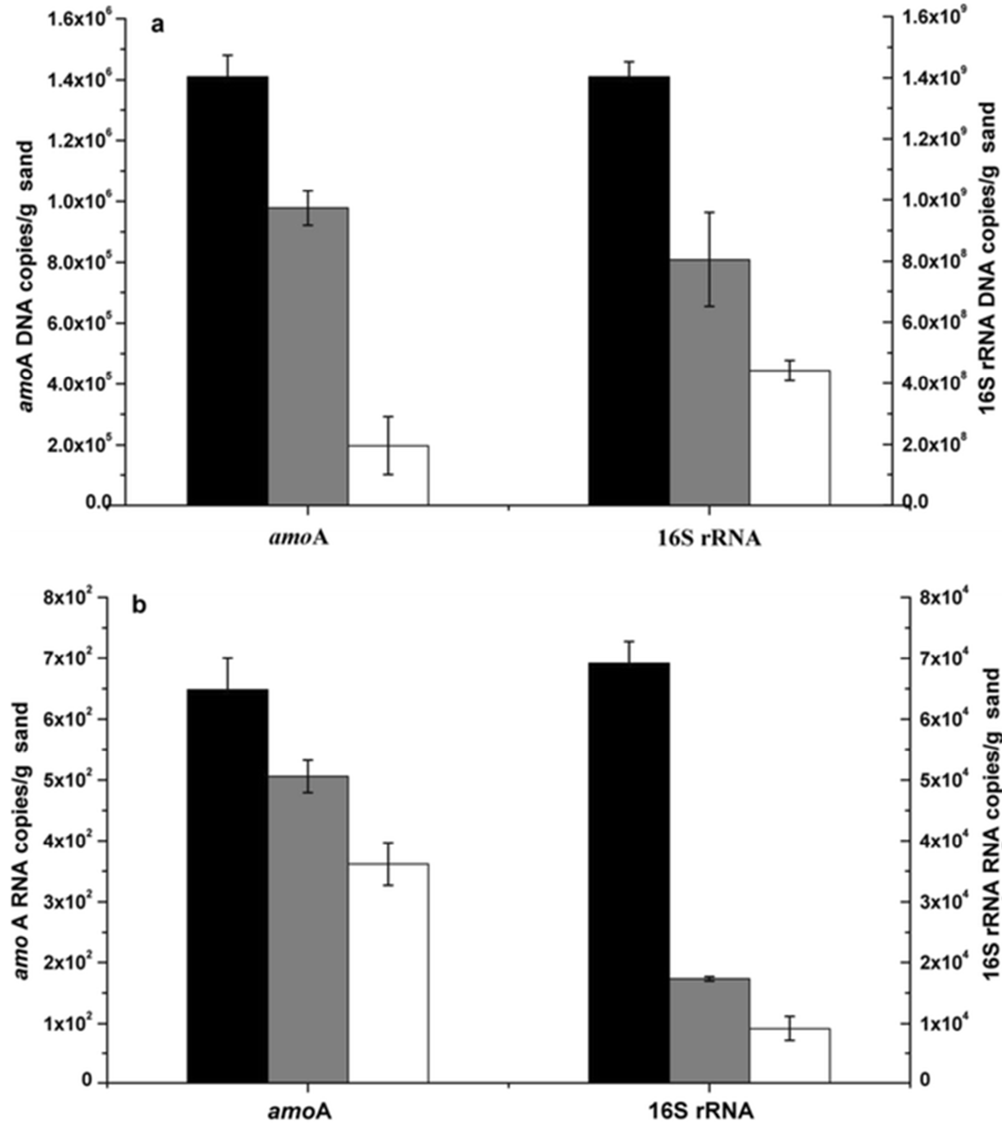

Fig. 3 DNA (**a**) and RNA (**b**) copy numbers for *amo*A of AOB and 16S rRNA of total bacterial in top sands (unharvested (*black*), harvested (*gray*), and cleared (*white*)). *Error bars* represent standard error ($n = 3$)

At low temperatures, the total bacteria copy numbers in unharvested wetlands based on the 16S rRNA gene was $1.41 \pm 0.04 \times 10^9$ copies/g sand, which was 1.7 and 3.1 times that of the harvested and cleared units, respectively (Fig. 3a). The results indicated that the winter removal efficiency corresponded with the microbial abundance. Similar trends were observed for the microbial activity during winter (Fig. 3b). The total bacterial activity was indicated by the RNA copy numbers of 16S rRNA; the activity was highest in the unharvested wetlands

($4.75 \pm 0.02 \times 10^4$ copies/g), followed by the harvested wetlands ($1.19 \pm 0.03 \times 10^4$ copies/g). Minimal microbial activity was observed in the cleared wetlands ($0.63 \pm 0.14 \times 10^4$ copies/g). Other studies indicated that mRNA levels respond to several environmental conditions, such as ammonia supplementation, ammonia deprivation, carbon deprivation, and metal stress (Aoi et al. 2004; Radniecki et al. 2009; Wei et al. 2006). In the present study, both the microbial abundance and activity corresponded with the effluent removal efficiency. Higher bacteria abundance and enhanced microbial activity were observed in planted microcosms compared with the cleared ones. This result can be explained by the capacity of large plant root systems to support the attachment of microorganisms, increasing their populations relative to the unplanted wetlands (Brix 1997). Besides, the relative amount of root exudates secreted from the belowground tissue may also have a positive impact on the microorganisms (Duarte et al. 2005; Jensen et al. 2007; Munch et al. 2007).

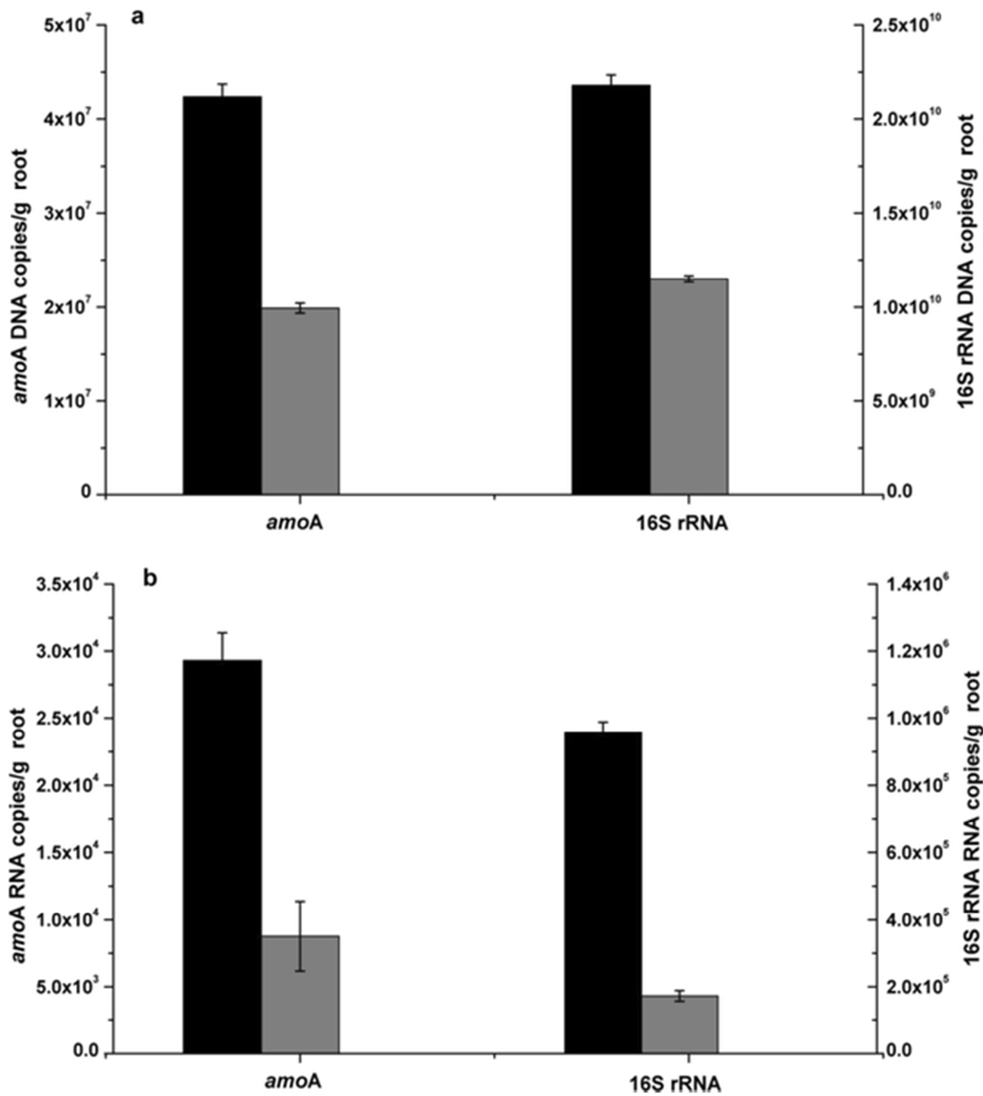

Fig. 4 DNA (**a**) and RNA (**b**) copy numbers for *amo*A of AOB and 16S rRNA of total bacterial on root surfaces (unharvested (*black*), harvested (*gray*), and cleared (*white*)). *Error bars* represent standard error ($n = 3$)

Figure 4 shows the number of DNA and RNA copies, respectively, on the root surfaces. Because the plants were completely removed in the cleared wetlands, no data could be shown for the cleared samples. The microbial abundance and activity in the sands were only 10 % that of the root surfaces (Figs. 3 and 4). The rhizome resources obtained from the roots possess enhancing effects that can increase the microbial biomass in the rhizosphere (Stottmeister et al. 2003). Plant roots support high levels of microbial abundance and activity primarily because they are sites of oxygen release and root exudation (Bürgmann et al. 2005; Bais et al. 2006; Munch et al. 2007). Hence, the differences in the microbial abundance and activity on the root surfaces of plants cultured in the wetlands may be explained by the relative reduced oxygen and organic carbon (exudates) leakage from roots of harvested plants.

We also detected that the *amo*A gene of AOB could give some insights into the observed microbial differences. Our results showed that the *amo*A gene copy numbers indicative of AOB were $1.41 \pm 0.01 \times 10^6$copies/g, which is 1.4 and 7.2 times those of the harvested and cleared ones (Fig. 3a). The activity of the AOB was highest in the unharvested wetlands ($6.48 \pm 0.52 \times 10^2$ copies/g), followed by the harvested ($5.06 \pm 0.03 \times 10^2$ copies/g) and the cleared ($3.62 \pm 0.03 \times 10^2$ copies/g) ones (Fig. 3b). These results have several implications. First, despite the high oxygen concentration in the effluent of cleared microcosms, more aerobic bacteria and microbial activity were observed in the planted ones, most likely as a result of root leakage. Second, plants with harvested aboveground biomass seemed to have a negative effect on the abundance of aerobic bacteria probably due to a relative reduction in the amount of secreted exudates from the belowground tissue as compared to the unharvested plants. Moreover, the AOB were ten times more abundant with higher activity on the root surfaces of unharvested plants (Figs. 3 and 4).

Radial oxygen loss

The ROL rates were detected at different temperatures shown in Fig. 5. The results showed that the unharvested units had higher oxygen release rates than the harvested ones. The ROL values for the unharvested units changed from 0.59 to 0.05 μmol O_2/h/plant as the temperature decreased, and a change of 0.52 to 0.01 μmol O_2/h/plant was observed for the harvested ones. The aboveground biomass provides oxygen to the belowground tissue through the aerenchyma (Caffrey and Kemp 1991; Connell et al. 1999; Pedersen et al. 1998, Brodersen et al. 2014a). Most of the oxygen transported to the roots is of photosynthetic origin (Hupfer and Dollan 2003; Sand-Jensen et al. 1982; Sorrell and Dromgoole 1988), and the oxygen transport within the aerenchyma is mainly through diffusion (Borum et al. 2006; Schuette et al.1994; Sorrell and Dromgoole 1988). Thus, irradiance (Connell et al. 1999; Flessa 1994), biomass (Caffrey and Kemp 1991; Kemp and Murray 1986), leaf area index (Connell et al. 1999), and diffusion pathways (Connell et al. 1999) have been identified as ROL-determining factors. Our results showed that harvesting aboveground biomass before winter could decrease the oxygen released from roots. The differences were significant when

the average temperature was above 4 °C (9.43 ± 0.60 to 5.56 ± 1.78 °C, $p < 0.05$). Previous studies suggested that higher oxygen content in wetlands creates better conditions for microbial processes (Faulwetter et al. 2009). In the present work, we found that higher ROL rates created better conditions for aerobic bacteria (as shown in the Materials and methods section) during winter. Ingemann Jensen et al. (2005) studied the oxic microniches around the roots of the seagrass *Zostera marina* L. via planar optodes and microelectrodes. They found that the ROL rates are generally highest in the subapical region of roots and decrease proportionally with the distance from the root apex. Oxygen leakage maintains an oxic microzone around the apical root meristem, thereby creating spatiotemporal oxic microniches in the sediment around actively growing roots (Brodersen et al. 2014b). Thus, root surfaces have a better aerobic environment than non-rhizosphere sands. Our results show that more aerobic bacteria with higher activity were present on root surfaces, as compared with those on sands, which agrees with several previous studies (Gagnon et al. 2007; Iasur-Kruh et al. 2010; Wang et al. 2012b). These results confirm that plant ROL has an important effect on microbial abundance and activity during winter (Wu et al. 2011).

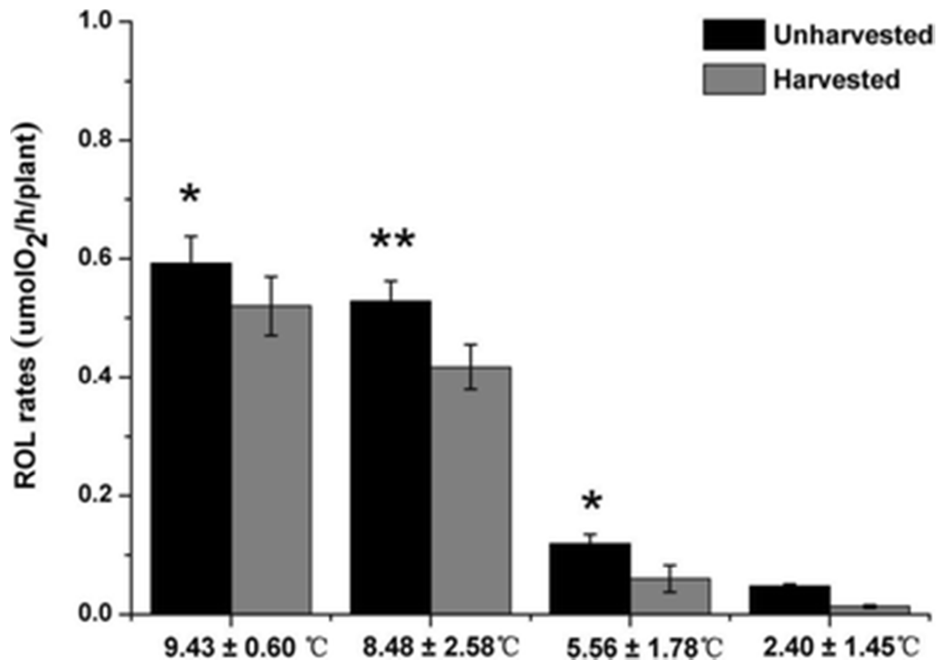

Fig. 5 ROL rates of unharvested and harvested plants at different temperature stages. $*p < 0.05$, $**p < 0.01$. *Error bars* represent standard error ($n = 3$)

A wide range of root oxygen release rates by using different methods and experimental conditions has been published for *P. australis*; these values range from 20 to 5120 mg O_2/m^2/day (Armstrong and Armstrong1990; Wiessner et al. 2006). Our results showed that 18.07 to 227.98 mg O_2/m^3/day was released from the unharvested wetlands during winter, and the harvested units released 5.1 to 200.16 mg O_2/m^3/day (Table 3). Given that 4.5 mg O_2 is required per milligram of nitrified N, ROL could theoretically supply 4.02 to 50.66 mg NO_3^--N/m^3/day of nitrified N (below 4 °C) in the unharvested wetlands, as compared with

1.14 to 44.48 mg NO_3^--N/m^3/day in the harvested ones. Hence, the different ROL rates caused by harvesting directly influenced the nitrified N in the planted wetlands.

Table 3 O_2 concentration (mg/m^3/day) provided from ROL of unharvested and harvested plants at different temperature stages (mean ± SD, $n = 3$)

Planted unit	Temperature stage			
	(9.43 ± 0.60 °C)	**(8.48 ± 2.58 °C)**	**(5.56 ± 1.78 °C)**	**(2.40 ± 1.45 °C)**
Unharvested	227.98 ± 17.57	203.13 ± 13.37	45.73 ± 6.08	18.07 ± 1.30
Harvested	200.16 ± 19.15	160.49 ± 14.56	22.95 ± 17.57	5.1 ± 0.10

The cleared wetlands had the lowest NH_4^+-N and COD removal efficiency without extra oxygen derived from plants via ROL. That is, the wetlands with macrophytes are obviously superior in terms of root zone oxidation to promote aerobic microbial processes (Stein and Hook 2005; Taylor et al. 2011). The oxygen in the cleared wetlands mainly came from reaeration. However, oxygen diffusion through water is approximately 10^4–10^6 times slower than through air (Drew 1981). Oxygen in planted wetlands can be obtained much faster from ROL by internal transfer via the air spaces in the plants (Armstrong 1978; Brix 1997). This phenomenon can explain the better growth conditions of planted wetlands for aerobic bacteria.

Furthermore, when the temperature was below 4 °C, the differences between unharvested and harvested wetlands were not obvious (2.40 ± 1.45 °C, $p > 0.05$). The ROL rates were almost zero at low temperatures regardless of whether the plants were harvested or not. The lower ROL observed from unharvested plants at low temperatures (i.e., below 4 °C) is due to a relatively lower rate of photosynthesis. As the light-independent reactions are highly temperature dependent and the rate of photosynthesis, therefore, increases in direct proportion to temperature until it reaches a temperature optimum for the given plant (Staehr and Borum 2011). Therefore, we speculated that plant roots were still active in winter but that their activity would be limited by low temperatures below 4 °C. In addition, all the effluent DO concentrations of the three kinds of wetlands were increased despite the decreased ROL rates with temperature reduction (Table 2). Figure 2shows that although the oxygen concentrations were increased, the removal efficiency decreased in all wetlands and differences still existed. Therefore, ROL had a potential influence on the microbial processes; its long-lasting and cumulative effects could not be ignored, even when the ROL decreased as the temperature was reduced to below 4 °C. Besides, the potential influence of root exudates secreted from the belowground tissue was also most likely important for the microbial processes and will be detected in detail in our future work.

Conclusion

Our results indicated that winter ROL significantly affect microbial abundance and activity in wetlands and thus played an important role in nutrient removal. Besides, late autumn

harvesting of aboveground biomass has negative effects on plant ROL, as well as the microbial population and activity during the following winter. Given the considerable ROL rates before the temperature was reduced to below 4 °C, it is suggested that harvesting should be carried out after the temperature was reduced to below 4 °C, rather than at the end of autumn.

Acknowledgments

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