Oxygen, pH, and Eh microprofiles around submerged macrophyte Vallisneria natans response to growing stages

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Abstract. The periphyton, attached to the surfaces of submerged plants, has important effects on plant growth and development in eutrophic waters. Periphyton complicates the microenvironment of diffusive boundary layer around submerged plants. We researched periphyton characteristics, oxygen (O_2), pH, and Eh microprofiles at various growing stages of *Vallisneria natans*. The results suggested that during the growing period of *V. natans*, O_2 concentration and pH decreased from 0 to 2 mm above the leaf surface, whereas the Eh increased. As *V. natans* grew, O_2 and pH gradually increased until they peaked during stable growing stages, while the Eh decreased. However, during the decline stage, O_2 and pH gradually decreased, and Eh increased. To summarise, O_2 and pH showed a unimodal pattern in response to the life cycle of *V. natans*, with the maximum levels during the stable growth stage and the minimum levels during the rapid growth and decline stages. Our study demonstrated that *V. natans* growth induced steep gradients in O_2 concentrations, pH, and Eh at the DBL by increasing the layer's thickness, macrophyte photosynthetic capacity, and periphyton biomass in eutrophic waters.

1. Introduction

Macrophytes provide the primary accessible surface area for the periphyton in eutrophic lakes. The periphyton attached on submerged macrophytes is an assemblage of microorganisms, inorganic particles, and organic detritus, forming a diffusive boundary layer (DBL) of periphyton–macrophyte association [1]. The DBL is important for macrophyte growth, nutrient transformation and biogeochemical cycles in eutrophic ecosystems [2, 3]. Oxygen (O_2) concentration, pH, Eh, and dissolved substances in the DBL differ considerably from the surrounding water, exhibiting steep gradients around the macrophyte surfaces [1, 4]. Characteristics of this DBL, including O_2, pH, and Eh levels, are affected by factors such as hydrodynamics, surface flux, concentration gradients, and periphyton surface structure. And light, temperature, plant growth stages and species of the associated macrophyte, also affect diffusive boundary layers indirectly, by affecting the flux of oxygen into and out of the leaf surface [2].

Of these factors, the effect of aquatic plant age on the DBL has been relatively understudied. We previously found that submerged plant *Potamogeton malaianus* leaf age had significant influences on the distribution of O_2, pH, and Eh at microscale, which exhibited the steepest gradient on adult leaves and the shallowest on old leaves [1]. Other studies have supported this pattern; for example, O_2

concentrations in the microprofiles of *Littorellauni flora* and *Potamogeton crispus* were significantly higher in early June than in late August. Moreover, as host plants grow, the periphyton is reported to increase in size, density, composition, structure, and species diversity, with senescent plants being the most densely colonised [5, 6]. However, we currently know little about how the characteristics of microenvironmental variables in the DBL may vary across different macrophyte growth stages. In fact, no study exists that investigates periphyton–macrophyte association across growth stages simultaneously with microprofile characteristics (specifically, measurements of O_2, pH, and Eh) around submerged plant leaves. This lack of data hinders a comprehensive understanding of the DBL's ecological function in eutrophic waters. We therefore require a detailed investigation of how plant growth affects microprofile dynamics and the structure of the periphyton–macrophyte association.

In this study, we used microsensors to measure the O_2, pH, and Eh microprofiles of submerged macrophytes at various growing stages. The aims of this study were to track the characteristics of O_2, pH, and Eh microprofiles, as well as the fine structure of the periphyton–macrophyte association, across the life cycle of submerged plants in eutrophic lakes. The consequent research results might help to make clear the processes and mechanisms of the DBL affecting nutrient cycle in eutrophic lakes and to fully understand the ecological remediation function and role of macrophytes in eutrophic waters.

2. Materials and methods

Vallisneria natans rhizomes were collected from Weishanhu District (34°46′ N, 117°08′ E) of Nansi Lake in China during November 2015. Sediment from the top 20 cm at the same site was gathered using a sediment corer. After removing large particles and plant/animal residue, the sediment was mixed uniformly and then stored until early March 2016, when it was used to plant *V. natans* rhizomes in the glass greenhouse (Linyi University, Linyi, China). Once the seedlings were 20 cm in height, during late April 2016, they were transplanted into high-density polyethylene bucket (Φ100 cm, H 120 cm) with 10-cm deep sediment. 260 seedlings each were planted in four such plant-sediment systems and incubated in the greenhouse. Water from the Nansi lake was injected slowly into the buckets, and water quality parameters (total nitrogen [TN], NH_4^+-N, NO_3^--N, NO_2^--N, total phosphorus [TP], chlorophyll a [Chl-a]) were measured after one week. We incubated *V. natans*in water that matched the quality of Weishanhu District. Nutrients, plankton and water were replenished regularly whenever depleted through sampling or evaporation. Monthly until the macrophytes died and decayed, we measured O_2, pH, and Eh microprofiles, periphyton, rapid light curves (RLCs), and the plant growth index of *V. natans*.

We analysed O_2, pH, and Eh microprofiles using microsensors (Unisense, Science Park Aarhus, Denmark), following methods modified from literature [1]. All experiments and calibrations were conducted at 20°C. To prepare the plants for measurements, we fastened macrophyte leaves on a plastic holder in a glass beaker. The experiments were carried out under controlled light from a fibre-optic halogen lamp (100 μmol photons$\cdot m^{-2}\cdot s^{-1}$) in laboratory. O_2 microelectrodes of 10 μm tip diameters were used to study O_2 microprofiles, and the O_2 microelectrode was mounted on a motor-driven micromanipulator (Unisense, Denmark) and connected via a Microsensor Multimeter to a personal computer. Linear calibration was performed using an O_2-free solution and air-saturated distilled water. The pH and Eh microelectrodes were calibrated and positioned according to the literature [1]. Measurements occurred before there was sufficient time for a gradient to develop. The gradients were measured with the periphyton intact.

Periphyton was scraped from a 10-g fresh weight sample of *V. natans* with a fine brush, and leaves were rinsed with sterile water. Subsequently, the water containing the periphyton was diluted with distilled water to 500 mL. Dry weight (DW), ash weight (AW), ash-free dry weight (AFDW), and Chl-a were measured according to previously described methods [1]. We also measured the rapid light curves (RLC) of *Vallisneria natans* with the pulse amplitude modulated fluorometer (Diving-PAM)

following methods from literature [1]. Data were analysed using Wincontrol v2.0 (Walz GmbH, Effeltrich, Germany).

Before statistical analyses, all data were tested for homogeneity and normal distributions of variance. The data of DW, AW, AFDW, and the Chl-a content of the periphyton were analysed with One-way ANOVA, with aquatic plant growth in months as the independent variable. If these results were statistically significant, the differences are experimented by a Tukey's honest significant difference (HSD) test with a confidence interval of 95%.

3. Results

The rapid growth stage for *V. natans* in this study spanned June, July, and August; the stable growth stage, September and October; and the decline stage, November and December. During the growing period of *V. natans*, the O_2 concentration and pH decreased from 0–2 mm above the leaf surface, whereas the Eh increased (figures 1a, 1b and 1c). The O_2 concentration and pH experienced a gradual increase as macrophytes entered the vigorously growing stage (figures 1a and 1b). This gradual increase continued with *V. natans* growth until both variables peaked during the stable growing stages. In contrast, during the decline stage, O_2 concentration and pH decreased as the plant grew older. Overall, O_2 and pH increases peaked in September, respectively reaching 552.7 $\mu mol \cdot L^{-1}$ and 9.4. This period coincided with the point at which macrophytes entered reproductive growth and growth slowed (figures 1a and 1b). Next, starting from October, O_2 and pH decreased gradually, dropping to their minimum values of 348.2 $\mu mol \cdot L^{-1}$ and 7.8, respectively, during December. Correspondingly, the spatial characteristics of O_2 and pH differed in June compared with December.

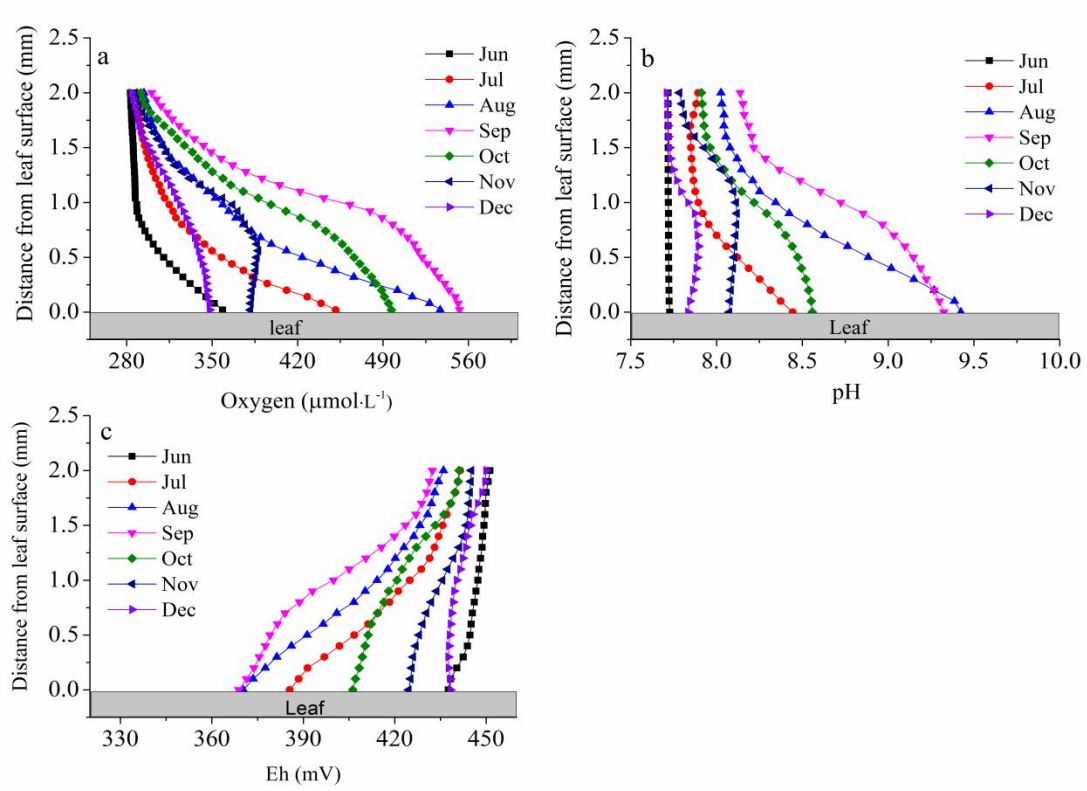

Figure 1. O_2, pH, and Eh microprofiles around *Vallisneria natans* leaves across months. O_2 microprofiles (a), pH microprofiles (b), and Eh microprofiles (c) were measured with microsensors as the macrophytes grew. Data points represent the means of triplicates.

Contrary to the microprofiles of O_2 and pH, the Eh markedly increased from 0–2 mm above the leaf surface, reaching its minimum value at the leaf surface. The decrease in Eh accompanied the entry of macrophytes into their rapid growth stage (figure 1c). Overall, Eh decreased gradually from June to September, reaching the lowest point (368.81 mV) in September (stable growing stage). Next, Eh increased gradually from October to December (decline stage). Neither December nor June experienced significant changes in Eh microprofiles, but the spatial characteristics of Eh differed in December compared with June.

The periphyton exhibited clear changes on the *V. natans* leaf surface during the macrophytes' life cycle (table 1). All measured variables except Chl-a (i.e. DW, AFDW, AW, and thickness) increased gradually from June to December, peaking in the latter month. Compared with their values in June, the DW, AFDW, AW, and thickness in December were 13.67, 14.17, and 13.52, 13.62 times higher, respectively. The proportion of AFDW in the periphyton increased gradually from June (18.81%), peaking (23.63%) in October, and then declining.

Table 1. Characteristics of periphyton on the *Vallisneria natans* across months.

Month	DW $mg \cdot cm^{-2}$	AFDW $mg \cdot cm^{-2}$	AW $mg \cdot cm^{-2}$	Chl-a $\mu g \cdot cm^{-2}$	Thickness μm
Jun	0.31 ±0.02 a	0.06 ± 0.01 a	0.25 ±0.01 a	1.03 ± 0.10 a	40 ± 3 a
Jul	0.59 ± 0.04 a	0.12 ± 0.01 a	0.47 ± 0.03 a	2.39 ± 0.22 b	77 ± 5 b
Aug	0.99 ± 0.07 b	0.21 ± 0.01 b	0.78 ± 0.01 b	3.26 ± 0.30 b	128 ± 9 c
Sep	1.67 ±0.08 c	0.37 ± 0.02 c	1.30 ± 0.07 c	6.31 ± 0.62 c	217 ± 15 d
Oct	2.32 ± 0.22 d	0.55 ± 0.04 d	1.77 ± 0.15 d	10.65 ± 0.83 d	330 ± 20 e
Nov	3.32± 0.27 e	0.71 ± 0.05 e	2.60 ± 0.22 e	15.13 ± 1.24 e	430 ± 30 f
Dec	4.24 ± 0.36 f	0.85 ± 0.06 f	3.38 ± 0.25 f	15.97 ± 1.27 e	550 ± 33 g

Notes: Different lowercase letters indicate that there is a significant difference.

The RLC, the curve of electron transfer rate changing with light intensity, contains information on physiological flexibility with which a plant can adapt its photosynthetic apparatus to rapid changes of light intensity. It allows insight into characteristics of actual photosynthesis of plant. Hence, RLC can appraise the photosynthesis capability of plant leaves. The RLC of *V. natans* leaves differed significantly across various months (figure 2), indicating that the leaves' photosynthetic capacity experienced monthly fluctuation Photosynthetic capacity increased gradually as *V. natans* grew, and reached its maximum in September (the stable growth stage), with a maximum potential rate of electron transport (ETR_{max}) of 34.85 μmol $electrons \cdot m^{-2} \cdot s^{-1}$. Starting from October, photosynthetic capacity then gradually decreased, reaching the lowest point in December (the decline stage), with an ETR_{max} that was 25.82% of the stable growth stage. The RLC patterns of variation were similar to patterns observed for O_2 and pH (figures 1a, 1b and 2).

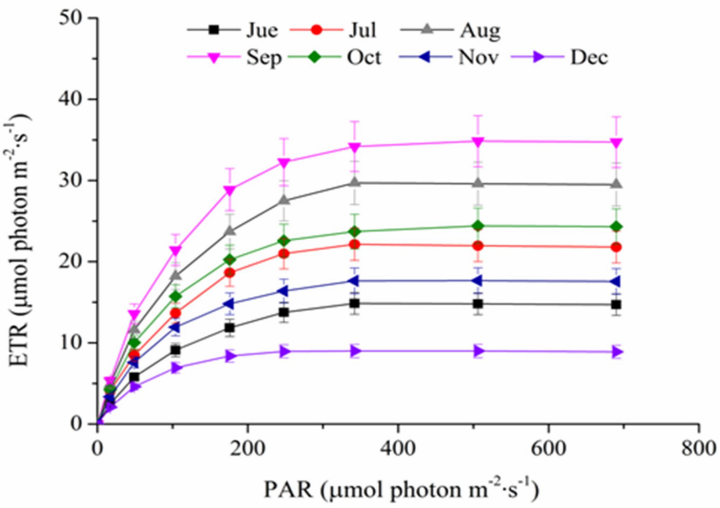

Figure 2. Rapid light curves of *Vallisneria natans* across months. Data points and vertical bars represent the mean of triplicates and standard errors, respectively. (photosynthetically available radiation, PAR; electron transport rate, ETR)

4. Discussion

Our results demonstrated that stages in the macrophyte life cycle could diversify O_2, pH, and Eh microprofiles (figures 1 and 3). The patterns of O_2 and pH values presented a unimodal pattern, being lower during the plant's rapid growth and decline stages, and higher during the stable growing stage (figure 3). Variation in the Eh was the opposite of the O_2 and pH patterns. These outcomes can be explained by the observation that while cell division and metabolism were quite fast during the rapid growth stage, photosynthetic capacity was also relatively weak due to low chlorophyll content in the young plant. Thus, the macrophyte exerted little effect on O_2, pH, and Eh microprofiles during this time.

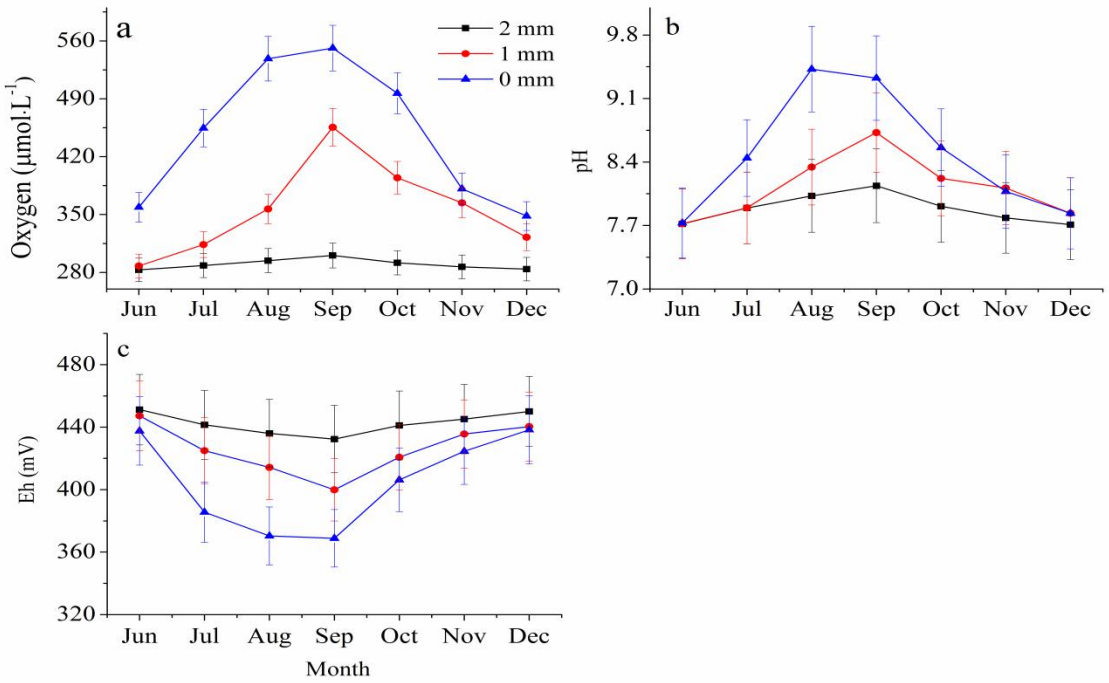

Figure 3. Magnitude of O_2, pH, and Eh at different distances from the *Vallisneria natans* leaf across months. O_2 concentration (a), pH (b), and Eh (c) values were measured with microsensors at distances of 0, 1, 2 mm above the macrophyte leaf surface. Data points and vertical bars represent the means of triplicates and standard errors, respectively.

However, once the maximum photosynthetic capacity was reached at the stable growth stage, *V. natans* was capable of exerting a powerful influence on O_2, pH, and Eh microprofiles. We therefore propose that the macrophyte life cycle affects O_2, pH, and Eh microprofiles via altering photosynthetic capacity. Different growth stages may also affect the distributions of CO_2, phosphates, nitrates, and microbes in the DBL. If these connections can be supported with future empirical research, it should provide new clues for the control mechanism of hydrophytes on nutrient cycle in eutrophic lakes.

Our data also support the idea of the periphyton being a major factor affecting O_2, pH, and Eh microprofiles, although the strength of the effect is strongly linked with the macrophyte life cycle. This link exists because periphyton accumulation and loss naturally accompanies macrophyte growth. During the rapid growth stage, *V. natans* was reported to continuously produce new tissue and slough off old tissue, which results in the easy detachment of the periphyton [6]. Additionally, previous research has also shown that the periphyton was inhibited by the phenolic acid secretions of *V. natans* during the rapid growth stage. Therefore, the periphyton was very sparse during this period in the macrophyte life cycle and did not exert any obvious effect on O_2, pH, and Eh microprofiles (table 1).

However, during the stable growth stage (September–October), *V. natans* biomass and physiological activity peaked, increasing the amount of microorganisms attached to the leaf surface. In turn, these microbes continuously secreted extracellular polymeric substance, which allowed more microorganisms and inorganic particles to adhere. As a result, the periphyton grew thicker and more biologically complex (table 1), with microbes actively modifying the colonised plant microenvironment. Thus, O_2, pH, and Eh gradients became significantly steeper due to the thicker periphyton and the high physiological activity of *V. natans* (figure 1). These changes in O_2, pH, and Eh increased the suitability of the external environment, allowing small microbial colonies to grow and eventually merge, supporting the dense periphyton [7].

Finally, during the decline stage, *V. natans* physiological activity decreased substantially, leading to a reduction in the fluctuation intensity of O_2, pH, and Eh. As a result, the distribution of O_2, pH, and Eh became dominated by the thick periphyton, rather than by the macrophyte.

To summarise, the O_2, pH, and Eh microprofiles measured in this study were the combined outcome of macrophyte growth stages and changes to the periphyton. We note that our results are supported by data from *Potamogeton crispus* in Almind Lake of Denmark, which demonstrated variations in O_2 microprofiles that are consistent with this study [2].

We found that the DBL microenvironment became more complex in response to submerged macrophyte growth and periphyton increases. The thickness of the DBL is positively correlated with highly eutrophic water. Moreover, the DBL can hinder soluble substance transport to the macrophyte, thus slowing macrophyte growth or even accelerating macrophyte decline. Therefore, research on O_2, pH, and Eh microprofiles around submerged macrophytes at various stages in their life cycle reveal more information about the DBL microenvironment, providing significant insights into macrophyte management and nutrient cycling in eutrophic waters.

5. Conclusion

Our results provide evidence that macrophyte growth stages induced steep gradients inO_2 concentrations, pH, and Eh of eutrophic waters, via increasing DBL thickness, macrophyte photosynthetic capacity, and periphyton biomass. We advocate more studies on how soluble substances and microbes are distributed or transformed in microprofiles, because the resultant data are necessary to understand how the DBL surrounding macrophyte leaves regulates nutrient cycling in eutrophic, polluted waters.

Acknowledgments

This research was supported by National Natural Science Foundation of China (41603071 and 41173078), Introduction of Talent Research Funding (LYDX2016BS074) and National Undergraduate Innovation and Entrepreneurship Training Program (201610452018).

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