Spatio-temporal patterns of redox potential diffusive boundarys around Potamogeton crispus leaves and stems

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Abstract. To understand functions of a diffusive boundary layer around submerged macrophytes, the redox potential (ORP) around the leaves and stems of Potamogeton crispus were measured using a microsensor. Periphyton attached on macrophytes was analysed with the standard method. Results showed that significant spatio-temporal variations of ORP existed around leaves of P. crispus. In a vertical direction (within 2 mm), ORP significantly decreased with reducing distance from the leaf /stem surfaces and reached a minimum (428.51 mv) at the leaf /stem surface. The ORP microprofile was steepest around the mature leaf at the middle shoot and that of the senescent leaves at the basal shoot was relatively flat. At the temporal scale, the ORP microprofile was steepest at the stable growing stage, and those of the seedling stage and declining stage were relatively flat. Periphyton attached on P. crispus became dense gradually upon macrophyte entering growth stages. The results indicated that characteristics of ORP microprofiles were mainly affected by macrophytic physiological characteristics and periphyton synergistically.

1. Introduction
In eutrophic shallow-water ecosystems, submerged macrophytes potentially play an important role in improving the water environment, and they often provide most of the accessible surface areas for attached periphyton affecting nutrient removal, partly due to the physical trapping of particles, plant uptake, and/or by modifying biogeochemistry [1]. The interface between the submerged macrophyte surfaces and water forms a unique diffusive boundary layer (DBL), because submerged macrophytes are not neutral substrata for periphyton. The microenvironment in DBL is heterogeneous due to the complex composition of periphyton, and to the host plant with biological activity. Understanding the environmental characteristics of DBL is the prerequisite for revealing various ecological functions of DBL. Previous researches suggest that dissolved oxygen (DO) concentrations and pH in DBL around submerged macrophytes Potamogeton crispus and Potamogeton malaianus showed significant spatio-temporal variations [2-4]. Preliminary studies seldom investigate the redox potential (ORP) in DBL around submerged macrophytes, hence little is known about the spatial and temporal patterns of ORP in DBL, which hinders understanding the full functions of DBL. So, in this study, a worldwide species Potamogeton crispus in eutrophic waters was selected. Using a microprofiling system equipped with microsensors, we analyzed the spatio-temporal patterns of ORP at a micro-scale (µm-mm).

2. Materials and methods
Submerged macrophytes Potamogeton crispus were collected from the trophic water of the Yihe river (E 118.32°, N 35.11°), in China. There are plenty of inorganic nutrients in the Linyi urban section of the Yihe River. Mature leaves were covered by thick periphyton layers due to high nutrient availability.
and suspended solids. Ten intact, healthy plants were collected for measurement of ORP microprofiles and periphyton at the seedling stage, rapid growing stage, stable growing stage, and declining stage, respectively, from March to June in 2015.

An intact Potamogeton crispus was placed in a aquarium of 5000 mL river water. The plant was fixed firmly on an agar plate (4%, w/w), which was then placed in a thermostatted water tank (20 °C). Experiment were carried out under controlled quantum flux densities 200 µE m-2 ·s-l, provided with a halogen lamp through fibre optics [2]. The ORP was determined by a microelectrode with 10 µm in tip diameter, which was combined with a Ag-AgCl reference macromicroelectrode (Unisense, Denmark). The microelectrode was calibrated linearly by redox buffers under the laboratory condition [2], and it was moved vertically by a motor-driven micromanipulator, which could be read at 1 µm precision. To avoid possible disturbance by the moving electrode, only when the electrode was moved down were microprofiles measured.

Periphyton on the P. crispus surfaces was collected on GF/C filters. The filters were dried (105 °C, 24 h) after which the dry weight (DW) was measured, and the filters were then heated at 550°C (4 h) in a muffle furnace (SX3-4-16), after which the ash weight (AW) was determined. The ash free dry weight (AFDW) was the difference between the DW and the AW [2]. The chlorophyll-a (Chl-a) level was measured using the standard method (China EPA).

Univariate data analysis was performed using SPSS for Windows version 17.0. The periphyton ash weight, ash free dry weight, dry weight, chl-a of, ORP at various growing stages, and that of different parts of macrophytes were analyzed using ANOVA. When the difference was significant, the Tukey's honestly significant difference test (Tukey's HSD) is used (p<0.05).

3. Results

3.1. Characteristics of ORP microprofiles at different temporal and spatial scales

In a vertical direction from the leaf surface within 2 mm, ORP reduced significantly with decreasing distance from leaf surfaces. On a temporal scale, ORP in the microprofiles changed significantly with highly diverse patterns among the various growing stages in the entire life cycle of macrophyte (figure 1). The variation of ORP was flat at seedling stages, and its amplitude increased as the plants grew. The ORP gradient was the steepest with decreased minimum (428.51 mv) at stable growth stages. Whereas, ORP gradients had spatially significant differences at declining stages due to thick periphyton.

On a spatial scale, ORP microprofiles showed highly diverse patterns among different parts of individual mature plants (figure 2). ORP microprofiles around apical leaves (younger leaf) at the shoot apex varied slightly, and those of the leaves (mature leaf) at the middle section of shoot were steepest. ORP microprofiles around stems at shoot middle section and basal leaves (senescent leaf) at the shoot decreased slightly when entering the dense periphyton layer, and were minimum at the surface of stems and leaves. And that, the periphyton exerted a significant effect on the ORP distribution (figure 2). The ORP amplitude of fluctuations obviously decreased fllowing periphyton removed.
Figure 1. ORP microprofiles around *Potamogeton crispus* leaves during different growth periods. Three profiles were measured at different points on *P. Crispus*. A distance of 0.0 indicates the leaf surface.

Figure 2. ORP microprofiles around different parts of *Potamogeton crispus*. A distance 0.0 indicates the macrophyte leaf surface.

3.2. Characteristics of periphyton at different temporal and spatial scales

The periphyton attached to *P. Crisps* leaf surfaces showed obvious dynamics during the life cycle (Figure 3a). Dry weight (DW), ash-free dry weight (AFDW), ash weight (AW), total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), and thickness of periphyton all increased significantly with the growth of *P. Crispus*. Periphyton increased significantly in the order of: apical leaves, stems, middle leaves, and basal leaves.
4. Discussion

4.1. Correlations between ORP and periphyton in a diffusive boundary layer
ORP indicates the tendency to receive or donate electrons. It is an important index of the natural aquatic environment affected by many biotic and abiotic factors [2, 5]. A correlation analysis was conducted between ORP and periphyton in the diffusive boundary layer around P. Crispus, in order to study the mechanism of ORP change (Figure 4). Data in the P. crispus life cycle demonstrated a negative correlation between ORP at leaf surface and periphyton indices such as DW, AFDW, AW, total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), and thickness of periphyton. However, the relatively low relevant coefficient (in the range 0.415 to 0.835) could be caused by a number of factors, as ORP was a combination of various reduction and oxidation [2]. The ORP at leaf surface increased and ORP gradients flattened following the removal of periphyton, possibly because of the diffusion resistance of oxygen decrease, and weakening micro-environment oxidation. Thus, dense periphyton increased the heterogeneity in DBL around leaves and stems.

4.2. The implications of ORP spatio-temporal patterns in DBL for aquatic ecosystems
There are usually only a few millimeters in DBL thickness, with biologically complex periphyton. The physiological activity of host plants made the environment more heterogeneous, which creates many difficulties for relevant research. At present, characteristics of the microenvironment in DBL, especially ORP, are not well understood. The growth of submerged macrophytes associated with the improvement of water quality is perhaps another reason for the significant variations of ORP microprofiles around P. crispus. In addition, the morphological and physiological complexity of P. crispus can provide a greater diversity of microhabitats for microorganisms to live in. Our previous study showed that oxygen produced via macrophyte photosynthesis could diffuse into bulk water. Therefore, the aerobic oxygen microregion formed at the surface of stems and leaves, and the anoxic oxygen microregion formed within periphyton due to aerobic organic matter decomposition [2,6,4], could be the main driving forces of ORP variation. The heterogeneous oxidation-deoxidation microenvironment may help to transform nutrients, and to regulate the nutrient cycle in aquatic ecosystems, and thus to alleviate lake eutrophication [1].
In the study, we analyzed firstly the spatio-temporal patterns of ORP microprofiles, revealing a heterogeneous oxidation-reduction microenvironment in DBL at the micron scale. The spatial patterns of ORP microprofiles were revealed via studying different parts of a whole plant, and the temporal patterns of ORP microprofiles were revealed via studying different growing stages within macrophyte life cycles. The results indicate that the growth status of submerged macrophytes have a significant impact on ORP microprofiles. Our results provide new information for exploring DBL functions of submerged macrophytes in eutrophic waters. We advocate more studies, focusing on micro-scale distributions of soluble substances (e.g. NH4+-N, NO3--N, NO2--N, PO43-, and CO2) and microorganism, because relevant results may help to understand the regulation of DBL on nutrient cycling in eutrophic ecosystems.

5. Conclusion
ORP microprofiles showed significant variations across different growing stages, with the steepest gradient at the stable growing stage affected synthetically by plant photosynthesis capacity, periphyton, light, and temperature. The steepest gradient of ORP occurred in the DBL of mature leaves on a whole macrophyte, and was affected by plant physiological activity and periphyton. The negative correlation between ORP level at leaf surface and periphyton indices provided new insights for understanding the function of a DBL.

References
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