



Oxygen and phosphorus dynamics in freshwater sediment after the deposition of flocculated cyanobacteria and the role of tubificid worms

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HIGHLIGHTS

- Algae deposition increased sediment O₂ uptake and decreased O₂ penetration depth.
- Algae deposition altered SRP flux, pore-water SRP profiles and P fractions.
- Tubificid worms transferred algae cells deeper in the sediment and mitigate their degradation.
- Worms enhanced the increase of SRP in pore water and loosely adsorbed P in sediment.

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ABSTRACT

Flocculation is a promising method for controlling harmful algal blooms; however, little is known about the effects of algae deposition by flocculation on benthic oxygen (O₂) and nutrient dynamics. In this study, we aimed to investigate the influence of cyanobacteria flocculation deposition on benthic O₂ and phosphorus (P) dynamics and the role of tubificid worms in the process. Chitosan and sediment particles were used to flocculate and deposit cyanobacteria cells onto lake sediment. The impulse deposition of algal flocculation degraded the deposited algal cells, which decreased the O₂ penetration depth in sediment and increased the O₂ uptake rate. Algae deposition also increased the soluble reactive P (SRP) in pore water and loosely adsorbed P in sediment, and changed SRP flux. Tubificid worms transported algal cells deeper into the sediment, mitigated their degradation, and altered the O₂ penetration depth, but not the O₂ uptake rate. Tubificid worms enhanced the increase in pore-water SRP and loosely adsorbed P in sediment. Therefore, the deposition of algal flocculation modifies the benthic O₂ and P dynamics, and tubificid worms can mitigate or enhance some of these processes.

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1. Introduction

Harmful algal blooms (HABs) are increasing world-wide due to anthropogenic nutrient enrichment [1,2]. They affect public health, tourism, fisheries, and ecosystems because they can lead to adverse tastes and odors and the presence of toxic materials [3,4]. Thus, determining methods for controlling HABs has become a challenging aquatic science task. Several technologies have been studied, including flocculation [5], coagulation–flotation [6], oxidation [7], and ultrasonic irradiation [8]. Of these control methods, flocculation is popular because it can quickly flocculate algal cells for

deposition onto sediment. For example, modified soils [9], sediments [10], sands [11], and mineral materials [12] have been verified to mitigate algae blooms effectively through the flocculation of algal cells. Chitosan-modified soil and sediment are able to quickly flocculate and deposit algal cells, and more than 90% algal cells were removed in 1 h using 1 mg chitosan and 10 mg soil or sediment in 1 L lake water containing 4.86×10^9 *Microcystis aeruginosa* cells [9]. Take the price of chitosan in China for example, 1 kg chitosan is about 25 USD and it is able to dispose 1000 m³ water; and sediment and soils are able to be obtained locally, so the cost is low using chitosan modified soil and sediment to flocculate algal cells. This method has been used in a small bay in Lake Taihu, China [13].

Previous studies on algal flocculation have focused on algae removal efficiency, whereas little research has focused on the influence of algal flocculation on benthic oxygen (O₂) and nutrient dynamics. However, natural algae or other organic matter can influence benthic element dynamics. For example, O₂ penetration depth

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was decreased and volume-specific O₂ consumption was increased by intense sedimentation during a seasonal algae bloom [14]. In addition, algal bloom deposition increased sediment respiration and altered the benthic nitrogen and phosphorus (P) dynamics [15,16]. The deposition of artificial algal flocculation is an imposed organic impulse for sediment. Will this artificial intense deposition influence the benthic O₂ and nutrient dynamics?

Benthic animals live in or on the sediment surface and are an important constituent of the benthic sediment ecosystem. In addition, the activities of benthic animals alter the sediment matrix and solute exchange across the sediment–water interface (SWI) [17]. Benthic animals can mix sediment particles [18], alter sediment stratification [19], and increase sediment porosity [20]. Their movements in sediment produce the heterogeneity of O₂, pH, and microbes [21,22]. Moreover, organic matter degradation [23,24], nutrient cycling [25–27], O₂ dynamics [28], and ferrous iron concentrations [29] are altered by benthic animals. Benthic animals can burrow through the deposited flocculation layer [30] and alter the degradation of pulse-settled organic matter and nutrient fluxes across the SWI [31]. In addition, algal flocculation has been verified to influence benthic animal community structure and diversity after 11 months [32]. Thus, the questions of whether benthic animals will be influenced by the deposition of algal flocculation in short time and whether benthic animals will influence the degradation and survival of deposited algae need to be studied in detail.

Phosphorus plays an important role in freshwater eutrophication and HABs [2,33]. Therefore, the control of P input is critical for the prevention of eutrophication in freshwater ecosystems [34]. In addition, the release of P from sediment to overlying water can influence algae development [33]. Oxygen is a central molecule for global element cycling and plays a key role in P cycling [28]. In freshwater ecosystems, cyanobacteria are the most common algal taxa that induce HABs [3]. In the present study, we aimed to investigate the influence of cyanobacteria flocculation deposition on benthic O₂ and P dynamics. The role of tubificid worms in the process was examined simultaneously because they accumulate in high densities in eutrophic aquatic ecosystems.

2. Materials and methods

2.1. Field sampling

Lake Taihu is a eutrophic lake in Eastern China, with an area of 2338 km². The cyanobacteria blooms in Taihu have received increasing attention in recent years because this lake is essential to the local fishery, water supply, environment, and tourism [1,35]. On July 9, 2012, sediment cores and lake water were sampled at the Dapu River estuary (31°18′19.1″ N, 119°55′58.2″ E) in western Taihu. Sediment cores were collected using plexiglas tubes (11 cm I.D., 50 cm long) and an 11 cm × 50 cm gravity corer. In addition, lake water was collected in plastic barrels.

On August 2, surface sediment was collected by a Petersen Grab sampler from the same site and was screened with a 0.5 mm net to collect the tubificid worms (*Limnodrilus hoffmeisteri*) for this experiment. Cyanobacteria cells were collected along the shore of the Dapu River estuary using a plankton net on August 28. All samples were transported to the laboratory immediately after sampling.

2.2. Microcosms

In the laboratory, the top 12 cm of each sediment core was sectioned into 0–4 cm, 4–8 cm, and 8–12 cm portions; the same portions from different cores were pooled together. Each pool was sieved using a 0.6 mm mesh to exclude macroinvertebrates and large particles and was then homogenized with a dough mixer. The

sediment pools were transferred into 24 Plexiglas tubes (11 cm I.D., 17 cm long) according to their original sequences and depths. Lake water was added to the sediment surface in each microcosm using intravenous needles, resulting in 24 microcosms with 12 cm of sediment and 5 cm of water. The microcosms were randomly separated into three groups with eight replicates. Three groups were assigned as the control treatment (no algae or tubificid worm addition, C), algae treatment (only algae addition, A), and algae and tubificid worm treatment (both algae and tubificid worm addition, A+T). The three microcosm groups were transferred to three corresponding water tanks and submerged in lake water. The water in each tank was aerated by a mini aerator to maintain O₂ saturation. The microcosms were pre-incubated for 3 weeks before the tubificid worms were introduced.

2.3. Experimental design

For each treatment, three microcosms were selected randomly for the measurement of O₂ uptake rate and soluble reactive phosphorus (SRP) flux across the SWI, whereas the remaining five microcosms were used for pore-water sampling and for analysis of *chlorophyll a* (*chl a*) in the sediment. On August 3 and 4, O₂ uptake rate and SRP flux were measured separately. On August 5, 285 tubificid worms were added to each microcosm (30,000 ind. m⁻²) in the A+T treatment. The addition density of tubificid worms was based on their density in our sampling area in Lake Taihu [36]. All microcosms were then incubated for 24 additional days (until August 29). To investigate the influence of tubificid worms on sediment, O₂ and pH profiles were measured on August 24 using microsensors (Unisense, Denmark), and O₂ uptake rate and SRP flux were examined again on August 26 and 27.

On August 29, the A and A+T treatment microcosms were removed from the water tanks. A 30 mL concentrated cyanobacteria “solution” (the dry algal cells weighed 2.7 ± 0.04 g) was then gently dispersed into the overlying water of each microcosm. One milliliter of 1 g L⁻¹ chitosan solution and 10 mL of mud that contained 3 g of dry sediment from the field sampling site, were added to the overlying water with a gentle stir to flocculate and deposit the algal cells. After the sedimentation of flocculated algae, 15 mL of mud (same as above) was gently dispersed on the flocculation surface to prevent resuspension of the algae. All microcosms were returned to their original tank and carefully submerged into the lake water. This time point was defined as day 0 of the experiment. The following incubation experiment lasted for 80 days, with water replacement every two weeks; a mini aerator was used in each tank to supply a sufficient amount of O₂ to water.

During the experiment, the O₂ uptake rate was measured on days 6, 13, 20, 27, 41, 55, and 69, whereas the SRP flux was examined on days 7, 14, 21, 28, 42, 56, and 70. The O₂ and pH profiles were measured on days 2, 7, 14, 21, 28, 42, 56, and 70. Pore water was acquired using a minipeeper with a vertical resolution of 4 mm [37]. Three minipeepers were prepared at a time, deoxygenated under nitrogen, and then separately inserted into microcosms from the three treatment groups on days 6, 13, 27, 41, and 76. Three days later (on days 9, 16, 30, 44, and 79), the minipeepers were removed from the microcosms and carefully flushed with oxygen-free deionized water. Ferrous iron and SRP in the pore water were analyzed immediately. After the minipeeper was removed, the surface sediment (0–2 cm) was sectioned into layers and freeze-dried for analysis of the *chl a* content. For the first two time points, the sediment was sectioned at depths of 0–5 mm, 5–10 mm, and 10–20 mm; for the remaining time points, the sediment was sectioned into 10 layers with depths of 2 mm to illustrate the transportation of algae by tubificid worms. At the end of the experiment, one of the three microcosms used for O₂ uptake rate and SRP flux measurements was sectioned into 0–2 cm, 2–4 cm,

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