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## Fine-scale bioturbation effects of tubificid worm (*Limnodrilus hoffmeisteri*) on the lability of phosphorus in sediments

Musong Chen <sup>a, b</sup>, Shiming Ding <sup>a, \*</sup>, Ling Liu <sup>b</sup>, Yan Wang <sup>a</sup>, Xigang Xing <sup>b</sup>, Dan Wang <sup>a</sup>, Mengdan Gong <sup>a</sup>, Chaosheng Zhang <sup>c</sup>

<sup>a</sup> State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing 210008, China

<sup>b</sup> College of Hydrology and Water Resources, Hohai University, Nanjing 210098, China

<sup>c</sup> GIS Centre, Ryan Institute and School of Geography and Archaeology, National University of Ireland, Galway, Ireland

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## ABSTRACT

This study investigated the effects of tubificid worm bioturbation on the lability of phosphorus (P) in microcosm sediments. High-resolution dialysis (HR-Peeper) and two types of diffusive gradients in thin films (DGT) (Zr-oxide DGT and ZrO-Chelex DGT) were used to measure soluble P and Fe, and labile P and Fe at a millimeter spatial scale. The worm bioturbation promoted P release (up to 511% of the control) to the overlying water on the 6th day, but it was reduced compared to the control (up to 171% of the control) from the 22nd day to the 102nd day because of the adsorption by Fe(III) oxyhydroxides. The worm bioturbation reduced the pore water soluble P concentration up to 48% and the DGT-labile P concentration up to 29% of the control from a sediment depth of –10 mm to approximately –130 mm before the 22nd day of incubation due to worm ingestion of sediment particles. Two-dimensional measurements of DGT-labile P also showed a much lower concentration of labile P around the worm burrow. This effect disappeared on the 53rd and 102nd day. However, the soluble P and DGT-labile P decreased again up to 41% and 38%, compared to the control from the sediment depth of –20 mm and –10 mm to approximately –130 mm, respectively, on the 152nd day of incubation due to the adsorption by Fe(III) oxyhydroxides. Soluble Fe(II) and DGT-labile Fe did not show significant changes from the worm bioturbation on the 6th day, but decreased up to 31% and 47% of the control after the 6th day. The results that worm ingestion of sediment particles is a significant driver of soluble and labile P reduction in the sediments before the 22nd day. After that, soluble and labile P reduction was attributed to P adsorption by Fe(III) oxyhydroxides.

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### 1. Introduction

Feeding and burrowing activities of benthic macroinvertebrates have attracted great research interest because of how they influence the biogeochemistry of chemical elements, especially phosphorus (P), in aquatic systems. P is a key element in regulating water eutrophication and algal bloom (Correll, 1998; Schindler, 1977). Different macrozoobenthos species exercise different feeding and burrowing activities including biodiffusers, gallery diffusers, upward conveyors, downward conveyors, and re-generators (Kristensen et al., 2012; Zhang et al., 2010a). These

activities have different effects on oxygen penetration and P release from the surrounding sediments. For example, bivalves, as bio-diffusers, pedal-feed from the sediment and filter phytoplankton and seston from the overlying water (Boltovskoy et al., 1995; Reid et al., 1992). Their respiration decreases the oxygen penetration and redox state, increasing the release of soluble reactive phosphorus (SRP) by up to 116% (Chen et al., 2016). In contrast, chironomid larvae, as gallery diffusers, ventilate their burrows for respiration and feed on plankton (Walshe, 1947), causing bio-irrigation of the sediment adjacent to the burrows. One study found that this import of oxygen-rich water led to a decrease of the SRP concentration in the sediments by up to 59% (Chen et al., 2015).

Tubificid worms are upward conveyors found commonly in eutrophic lakes. They vertically burrow into sediments, feed head-down at depth, and then transport particles from the deep horizons

\* Corresponding author.

E-mail address: [smding@niglas.ac.cn](mailto:smding@niglas.ac.cn) (S. Ding).

to the sediment surface (Kristensen et al., 2012; Adámek and Maršálek, 2013). There are different theories about the role of tubificid worm bioturbation in releasing P. Some studies found that tubificid worm bioturbation promoted P release from sediments (Yao et al., 2011; Mermillod-Blondin et al., 2005; Zhang et al., 2010b, 2014), while other studies reported the opposite effect (Lewandowski and Hupfer, 2005; Heilskov and Holmer, 2001; Mortimer et al., 1999; Matisoff et al., 1985; Zhang et al., 2010a). Study results have also found differences in how these activities impact pore water P concentration. Zhang et al. (2010a) observed a negligible effect, while Matisoff et al. (1985) and Ding et al. (2011) reported that tubificid worm bioturbation decreased pore water P concentration. Therefore, more study is needed to understand the effects of tubificid worm bioturbation on the lability of P in sediments and the release of P to the overlying water.

Several studies have measured pore water P concentrations to investigate the effects of bioturbation on lability of P in sediments (Lewandowski and Hupfer, 2005; Lewandowski et al., 2007; Matisoff et al., 1985; Zhang et al., 2010a). Chen et al. (2015) found that SRP concentration is not a sufficient indicator to assess chironomid larvae bioturbation effects on sediment P, because the P adsorbed on minerals (such as  $\text{Al}(\text{OH})_3$ ) may provide a buffer for the pore water P once released from the bioturbation sediment particles. Instead, the sediment labile P concentration, measurement by a dynamically passive sampling technique called diffusive gradients in thin films (DGT), is a more sensitive indicator. This is because that the measured DGT-labile P not only includes pore water P, but also includes loosely bound P released from sediment solids (Ding et al., 2010). Meanwhile, the individual bodies of macrozoobenthos are small in size, often at a millimeter level. This requires a high resolution sampling technology to reflect the heterogeneous changes of P around the macrozoobenthos burrow. High-resolution Peeper (HR-Peeper) and DGT effectively satisfies this requirement (Davison and Zhang, 1994; Xu et al., 2012, 2013; Ding et al., 2010, 2011). Further, Ding et al. (2013) developed a high-capacity Zr-oxide DGT to measure labile P in sediments at a submillimeter, two dimension levels. This provides a reliable image of labile P distribution from bioturbation.

In this study, two types of DGT (Zr-oxide DGT and ZrO-Chelex DGT) and HR-Peeper were used to investigate the effects of tubificid worm bioturbation on the lability of P at the sediment-water interface (SWI) at the one dimension (1-D) and two dimension (2-D) levels. The 1-D concentration profiles of soluble and labile Fe in the sediment were simultaneously obtained, and the mechanisms behind the bioturbation effects are discussed.

## 2. Materials and methods

### 2.1. Preparation of HR-Peeper and DGT probes

Other studies have discussed the principles of HR-Peeper and DGT (Davison and Zhang, 1994; Ding et al., 2010; Xu et al., 2012, 2013). The HR-Peeper was used to measure soluble Fe and P in pore water at a 2.0 mm spatial resolution. The HR-Peeper probe was prepared based on Xu et al. (2012). In assembling the HR-Peeper, the chambers were filled with deionized water and covered sequentially by a 0.10 mm Durapore® PVDF membrane (0.45 µm pore size, HVLPO0010, Millipore) with an open area of a 1.8 cm × 15 cm plastic window.

Two DGT techniques were used in this study. The first one was the high-capacity Zr-oxide DGT to measure labile P in sediments at a submillimeter, 2-D level in combination with detections using surface coloration and computer-imaging densitometry technique (CID). The second technique was a ZrO-Chelex DGT to simultaneously measure labile P and labile Fe on a 1.0 mm spatial

resolution. Zr-oxide and ZrO-Chelex binding gels were prepared based on Ding et al. (2015) and Xu et al. (2013). When assembling the DGT probes, the binding gel was covered with a Durapore® PVDF membrane. All HR-Peeper and DGT probes were soaked in deionized water and deoxygenated with nitrogen for at least 16 h prior to deployment in the sediment.

### 2.2. Experimental microcosm set-up

Sediments used in the laboratory experiment were collected from Meiliang Bay of Taihu Lake (31°30'31.1"N, 120°10'31.0"E), the third largest freshwater lake in China. Meiliang Bay has been polluted by sewage discharges since the 1980s. This has resulted in water eutrophication and associated algal blooms. Ten sediment cores (11 cm in diameter, 40 cm in length) were collected from the bay using a gravity corer (11 cm × 50 cm, Rigo Co., Japan) on July 1, 2014. Samples of the overlying water were collected concurrently, using plastic barrels for laboratory incubation experiments. The tubificid worms were collected with a Peterson Grab (length × width × height = 36 × 20 × 15 cm) at the sampling site.

Each sediment core was sectioned at 2 cm intervals to 12 layers. The sediment layers at the same depth were composited and thoroughly homogenized. Sediments samples were then sieved through a 0.6 mm pore-size mesh to remove macrofauna and large particles. The 12 layers samples were then placed in 10 Perspex tubes (11 cm in diameter, 30 cm in length) according to their original depth. Afterwards, 5 of the original 10 sediment cores were placed in a tank, with an additional 45 cm thick layer of filtered lake water to submerge the sediment cores. The microcosms were pre-incubated for 16 days before introducing to the macrofauna. The water was maintained at 25 °C and aerated to maintain  $\text{O}_2$  saturation during incubation.

On July 16th, 2014, 16 days after sampling, 505 tubificid worms (*Limnodrilus hoffmeisteri*) about 0.7 mm wide and 25 mm long were added in one tank, with 101 worms in each of the five cores. Five cores in another tank served as the control without the addition of worms. The number of added worms was selected to replicate the population density at the sampling sites (10,633 individuals/m<sup>2</sup>). The microcosms were incubated under the conditions described above. The water was aerated for 5 min per each hour to maintain the oxic environment.

### 2.3. Sampling

On the 6th, 22nd, 53rd, 102nd, and 152nd day after the beginning of incubation, dissolved oxygen (DO) concentrations were measured using an oxygen microelectrode (OX-100, Unisense, Denmark). Afterwards, P flux across the SWI was calculated by comparing changing SRP concentrations over time in the overlying water, based on Zhang et al. (2010a). A 2 ml water sample was collected from each core using a pipette; samples were collected at 1 h intervals for 4 h. We sampled three portions of water in a core and analyzed respectively. The samples were immediately filtered through 0.45 µm cellulose acetate membranes for analysis.

In addition to these samples, the HR-Peeper probe was inserted along several worms posterior which stayed at the sediment surface into a single core for 48 h. At 24 h after insertion of the HR-Peeper, the Zr-oxide and ZrO-Chelex DGT probes were simultaneously inserted along several worms posterior into the same core and left for another 24 h. Prior to retrieving the probes from the core, the sediment and overlying water around each Peeper or DGT probe was sealed using a rectangular, hollow PVC. This prevented P and Fe diffusion to other cores through the hole formed by inserting the probes.

After retrieving the DGT probes, probe surfaces were rinsed with

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