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Journal of Environmental Sciences

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Environmental catalysis and materials

Influence of Chironomid Larvae on oxygen and nitrogen fluxes across the sediment-water interface (Lake Taihu, China)

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Abstract

The microscale distribution of oxygen, the nitrogen flux and the denitrification rates in sediment inhabited by chironomid larvae (*Tanypus chinensis*) were measured in eutrophic Lake Taihu, China. The presence of the chironomids in the sediment increased the oxygen diffusional flux from 10.4 ± 1.4 to 12.7 ± 2.5 mmol $O_2/(m^2 \cdot day)$. The burrows of the larvae represented "hot spots" and strongly influenced the nitrogen cycles and diagenetic activity in the sediment. The results indicate that the bioturbation effects of *Tanypus chinensis* chironomid larvae increased the capacity of the sediment as a sink for nitrate and a source for ammonium. Nitrate influx and ammonium outflux were increased 8.8 and 1.7 times, respectively. Under bioturbation, the amount of nitrate consumed was greater than the amount of ammonium released. The total denitrification rate was also enhanced from 0.76 ± 0.34 to 5.50 ± 1.30 mmol $N/(m^2 \cdot day)$. The net effect was that the bioturbated sediments acted as a net sink for inorganic nitrogen under direct and indirect bioturbation effects compared to the control.

Key words: bioturbation; chironomid larvae; oxygen; nitrogen; Lake Taihu

DOI: 10.1016/S1001-0742(12)60116-8

Introduction

As one of the most important energetically favorable electron acceptors for the oxidation of organic matter and other reduced chemical species such as NH_4^+, Fe^{2+}, Mn^{2+} and H_2S (Fenchel et al., 1998), oxygen regulates many important biogeochemical processes and has crucial implications for the biological and ecological processes of benthic communities (Fenchel, 1996; Rysgaard et al., 2001; Kristensen, 2000; Glud, 2008). The oxygen concentration in sediments depends on a number of processes, including diffusion, advection, bioturbation and the re-oxidation of reduced substances (Wenzhöfer et al., 2001). The oxygen penetration depth (OPD) below the sediment-water interface (SWI) is limited by higher levels of consumption and lower diffusion efficiency associated with the sediment (Jørgensen and Revsbech, 1985; Fenchel et al., 1998; Jørgensen and Boudreau, 2001; Wang et al., 2001). Most sediment-dwelling animals live below this thin oxide layer. They may not be able to withstand prolonged exposure to the ammonia or sulfide that is often present in deep and anoxic sediments (Miron and Kristensen, 1993; Levin et al., 2003; Sturdivant et al., 2012). As a consequence of their activities, these organisms introduce the overlying water into their burrows or the sediment for respiration (Fenchel, 1996; Wang et al., 2001; Wenzhöfer and Glud 2004; Jørgensen et al., 2005; Zorn et al., 2006), then the oxygen dynamics in the sediments are increased and oxic zones around their burrows are created (Koike and Mukai, 1983; Wang et al., 2001; Stief and de Beer, 2002; Jørgensen et al., 2005; Zorn et al., 2006; Zhang et al., 2011).

Solute transport across the SWI would also be enhanced under bioturbation. For example, it is estimated that the presence of amphipods in Antarctic sediments can increase the release of NH_4^+ up to 2 times by direct excretion or stimulation of vertical transport processes (Nedwell and Walker, 1995). In addition, several studies have shown that bioturbation activity can also increase the nitrate consumption and denitrification rates in sediment (Matisoff, 1995; Svensson and Leonardson, 1996; Laverock et al., 2011; Michaud et al., 2006).

Sediment has been hypothesized to act as a source or

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sink of inorganic nitrogen (Laverock et al., 2011; Fan et al., 2004), but a strong influence on solute transport across the SWI under bioturbation would change the source-sink states of the sediment. For example, the bioturbation effects of chironomid larvae, one of the most widely distributed and abundant groups of insects in freshwater environments (Pinder, 1986; Cai et al., 2011), were found to enhance nitrate (NO_3^-) consumption and denitrification rates (Mermillod-Blondin and Rosenberg, 2006; Risgaard-Petersen, 2004; Welsh, 2003; Svensson, 1997; Svensson and Leonardson, 1996; Michaud et al., 2006). More nitrogen was removed rather than being released to water due to the bioturbation effects of chironomid larvae. The bioturbated sediment acted as a more pronounced sink for inorganic nitrogen, whereas the control sediment acted as a source for inorganic nitrogen (Svensson, 1997). This source-sink transition under bioturbation would be beneficial because it would decrease the amount of nitrogen available to the primary producers in eutrophic lake (Qin, 2008; Conley et al., 2009; Xu et al., 2010).

Lake Taihu has suffered serious eutrophication in recent decades. Nitrogen has been shown to be one of the key factors responsible for harmful algal blooms in the lake (Xu et al., 2010; Conley et al., 2009). The larvae of *Tanypus chinensis*, one of the common chironomid species, dig burrows in the sediment and appear to have strong influences on the micro-topographic properties of the surface of the sediment. Therefore, they represent a good model-animal for bioturbation research. However, only limited bioturbation studies involving these species have been published in China (Zhang et al., 2010). Accordingly, we chose *Tanypus chinensis* larvae as the model animal for the present study.

The aim of this study was to investigate the bioturbation effects of *Tanypus chinensis* larvae on the oxygen distribution, nitrogen flux and denitrification rates in the sediment of Lake Taihu.

1 Materials and methods

1.1 Sampling location

Lake Taihu is a typical shallow freshwater lake in the middle to lower valley of the Yangtze River, China. It is the third largest freshwater lake in China, with a water surface area of 2338 km^2, an average water depth of 1.9 m, a maximum depth of 2.6 m, and a surface elevation of 3.0 m above sea level (Qin, 2008). Meiliang Bay, located in the northern part of Lake Taihu, is an important water source for Wuxi City and is a significant area for the surrounding region. Meiliang Bay has suffered harmful algal blooms in recent decades, due to rapid population growth, as well as rapid industrial and agricultural development (Chen et al., 2003; Qin et al., 2007; Guo, 2007). To study the effect of bioturbation on nitrogen and oxygen flux in heavily eutrophic regions, we performed an experiment in the
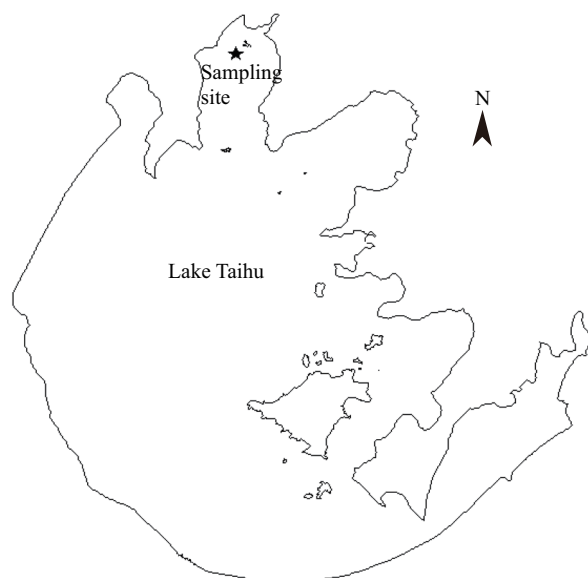
Fig. 1   Map of the study area and sampling site.

laboratory with sediment from a sampling site (**Fig. 1**, 31°30′31″N, 120°10′31″E) located in Meiliang Bay.

1.2 Sediment and fauna preparation

Sediment was collected in Plexiglas tubes (75 mm inner diameter, 30 cm long) with a gravity corer in December 2009. The top 12 cm of the core was sectioned at 2 cm intervals, and all intervals from the same depth were pooled together. All the sediments were sieved (0.25 mm, 60 meshes) to remove invertebrates, large detritus and algal aggregates, then homogenized in a stirred vessel and placed into the Plexiglas tubes according to their original depth. Following that step, the Plexiglas tubes were submerged in aquaria under circulating, aerated lake water at 20 ± 1°C for 4 weeks, on a 12-hr light/dark cycle. *Tanypus chinensis* larvae (ca. 10 mm long) were collected and were temporarily stored with a small amount of the original sediment under circulating, aerated lake water at the same temperature (20 ± 1°C).

Six Plexiglas tubes were divided into two treatments with three replicates each. The control treatment was set up without any organisms, for another test, ten chironomid larvae were added to each tube (2264 ind./m^2). The biomass is within the range normally found in eutrophic lakes (Svensson and Leonardson, 1996). The six cylinders were allowed to stabilize in aerated water for another 24 hr after the larvae were introduced. Before measurement of the oxygen profile, the overlying water was adjusted to reach a water height of approximately 12 cm (0.5 L). At the end of the experiment, all sediments were sliced into four layers (0–2, 2–5, 5–10 and 10–12 cm) and sieved (60 mesh) to collect the larvae and analyze the sediment characteristics. The sediment water content was calculated based on the weight loss after drying the fresh sediment to a constant weight at 60°C. The organic matter content

was determined by calculating the weight loss after heating the sediment samples at 550°C for 5 hr. Total nitrogen and total phosphorus were determined after extraction with an oxidizing reagent solution of NaOH-K$_2$S$_2$O$_8$ (Chinese SEPA, 2002).

1.3 Oxygen microprofiles

Oxygen microprofiles were measured with an oxygen microsensor (PreSens, Regensburg, Germany) with a small tip size (< 50 μm) and rapid response time (time to reach 90% of the final response (T90) < 1 sec). Oxygen microsensors have been frequently used to determine the oxygen concentration in sediment (Klimant et al., 1995; Irwin et al., 2007; Zorn et al., 2006). During profiling, the SWI was determined visually with the aid of a magnifying lens. The sensitivity of oxygen microsensors decreases over time during measurements. Therefore, a two-point linear calibration between oxygen-free solutions (saturated solution of Na$_2$SO$_3$, 0% air saturation) and air-saturated water (100% air saturation) was performed before each measurement. The measurements were arranged along horizontal transects. Each transect was 10 mm long and consisted of ten vertical profiles (vertical resolution: 20 μm, interval between two profiles: 1 mm). To study the spatial heterogeneity of the oxygen profiles, two-dimensional (2-D) isopleth graphs were plotted with Surfer 8.0 (Golden Software, Inc.).

1.4 Flux and denitrification

The molecular diffusion flux of oxygen is one of the principal parameters used to calculate the mineralization of benthic organic matter (Kristensen, 2000; Glud, 2008). This parameter can be calculated from O$_2$ micro-gradients measured in the diffusive boundary layer (DBL) (Jørgensen and Revsbech, 1985; Kiihl and Revsbech, 2001; Boudreau and Jørgensen, 2001).

$$J = -D(dC(z)/dz) \tag{1}$$

where, D (cm^2/sec) is the molecular diffusion coefficient of oxygen in water, C (mmol/L) is the oxygen concentration and z (mm) is the depth ($z = 0$ at the sediment surface). The value of D was taken from the study of Broecker and Peng (1974) and was recalculated for the appropriate *in situ* temperature using the Stokes-Einstein relation.

After the oxygen profile was measured, a flow-through system was constructed for the measurement of nitrogen flux and denitrification, according to the method of Mc-Carthy (2003). Rapidly, the tube was sealed with a piston equipped with a rubber O-ring to ensure that there was no headspace in the tube. Two Teflon hoses in the piston were used to supply the inflow and collect the outflow water. The flow-through speed was controlled with an ISMATEC Tubing pump with a constant speed of 1.44 L/day. The constant flow rate and the control of the temperature allow high levels of measurement stability and precision. A

known amount of ^{15}NO$_3^-$ was added to the inflow water to give a final concentration of 50 μmol/L (98 % Na^{15}NO$_3$). Incubation was performed for 48 hr. The sampling bottles were then filled with outflow water and allowed to overflow at least three volumes. ZnCl$_2$ (0.5 mL, 50%, m:V) was injected to inhibit bacterial metabolism. The sampling bottle was rapidly sealed with a glass plug and barrier film (Parafilm). The flux across the SWI was calculated according to Eq. (2):

$$F_n = (C_n - C_{n,0}) \times V/S \tag{2}$$

where, F_n (mmol/(m^2·day)) is flux, C_n (mmol/L), $C_{n,0}$ (mmol/L) are the concentrations in the outflow and inflow water, respectively, V (L/day) is the speed of tubing pump, S (m^2) is surface area of the tube.

Then denitrification was calculated from the production of ^{29}N (^{14}N^{15}N) and ^{30}N (^{15}N^{15}N) with a membrane inlet mass spectrometer (Nielsen, 1992; Kana et al., 1994; Svensson et al., 2001). The rates of denitrification of nitrate diffusing from the overlying water (D_w) were calculated from D15 and %^{15}NO$_3^-$, where %^{15}NO$_3^-$ was measured as the NO$_3^-$ increase in water occurring after the addition of ^{15}NO$_3$:

$$D_w = D15/\%^{15}NO_3^- \tag{3}$$

$$D15 = (^{14}N^{15}N) + 2(^{15}N^{15}N) \tag{4}$$

The rate of denitrification of nitrate produced by nitrification (coupled nitrification-denitrification, D_N) was calculated as the following equations (Nielsen 1992):

$$D_N = (D15 + D14) - D_w \tag{5}$$

$$D14 = D15 \times (^{14}N^{15}N)/2(^{15}N^{15}N) \tag{6}$$

2 Results

2.1 Visual observations

Tanypus chinensis larvae began to excavate sediment for burrow construction several minutes after they were introduced. All the larvae burrowed into the sediment within 12 hr. Their burrowing activities destroyed the initial surface of the sediment and clearly changed the surface's micro-topographic properties. Light colored oxidized burrow walls and black reduced sediment were clearly visible through the sides of the Plexiglas tubes (**Fig. 2**). The burrows averaged approximately 1 mm in diameter. No clearly visible color changes were observed in the control sediments.

At the end of the experiment, all of the sediments at different depths were sieved, with a 60 mesh sieve, to collect the larvae. All the larvae were recovered, and
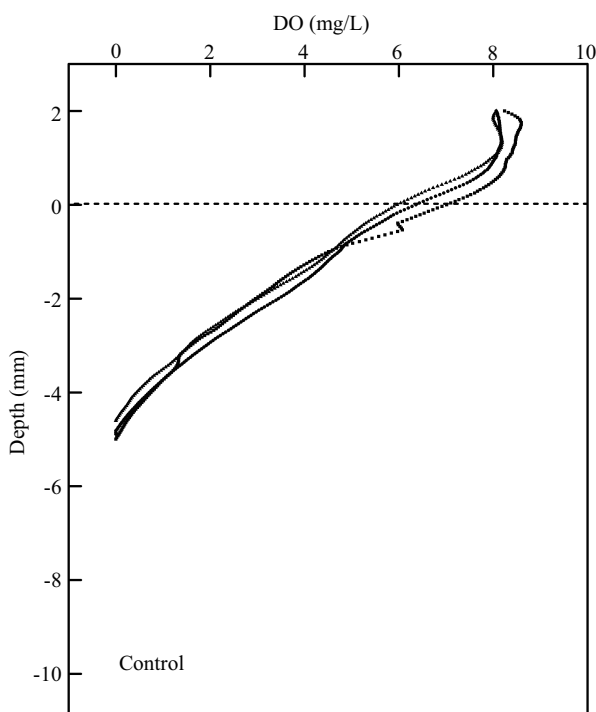
Fig. 2 Visual observations of the bioturbated core. Light-colored oxidized burrows along with black reduced sediments were clearly visible in the bioturbated core.

Table 1 Characteristics of surface sediment (0–5 cm deep, $n = 3$)

Feature	Control	Bioturbated
Clay fraction of particle size 0.02–4 μm (%)	14.6 ± 0.6	14.2 ± 0.7
Silt fraction of particle size 4–63 μm (%)	69.3 ± 4.2	71.4 ± 4.3
Sand particles of particle size 63–500 μm (%)	16.1 ± 0.8	14.3 ± 0.7
Ca (mg/g)	6.7 ± 0.1	6.517 ± 0.1

approximately 90% of the larvae were observed in the upper 5 cm of the sediment. There were no apparent differences in sediment characteristics among the different treatments (**Table 1**, T-test, $p > 0.05$).

2.2 Microprofiles of oxygen

When the microsensors entered the DBL (approximately 0.5 mm above the surface of the sediment), the DO began to decrease steeply (**Figs. 3** and **4**). The average OPD in the three control sediment cores was 4.50 ± 0.38, $4.48 \pm$

0.17, and 4.51 ± 0.19 mm respectively.

Because no apparent differences in the OPD were found among the three control cores ($p > 0.05$, T-test), only one of the cores was selected for further study of the 2-D oxygen distribution. The 2-D O_2 distribution in the control was uniform. In the control sediments, no sharp peaks of DO concentration were observed (**Fig. 5a**). In the bioturbated sediments, the OPD was enhanced up to 9 mm (**Fig. 4**). The DO concentration showed a sharp increase at 4–6 mm along the transect and reached a maximum at a depth of 7 mm (**Fig. 5b**). These findings showed that the chironomid larvae (and their burrows) facilitated the deeper penetration of oxygen into the sediments and strongly influenced the heterogeneous distribution of oxygen.

2.3 Oxygen, nitrogen flux and denitrification rates

The oxygen diffusive flux was calculated according to the O_2 micro-gradients measured with the oxygen microsensor (**Fig. 6**). In the control cores, the flux ranged from 8.6 to 13.7 mmol O_2/(m²·day), with a mean of 10.4 ± 1.4 mmol O_2/(m²·day). The presence of chironomid larvae increased the oxygen flux to values ranging from 7.9 to 17.9 mmol O_2/(m²·day), with a mean value of $12.7 \pm$

Fig. 3 Selected microprofiles in control sediments. Three repeated measurements of profiles showed that these profiles were reproducible and had a smoothly declining trend. The horizontal dashed line at 0 mm depth indicates the sediment-water interface (SWI).
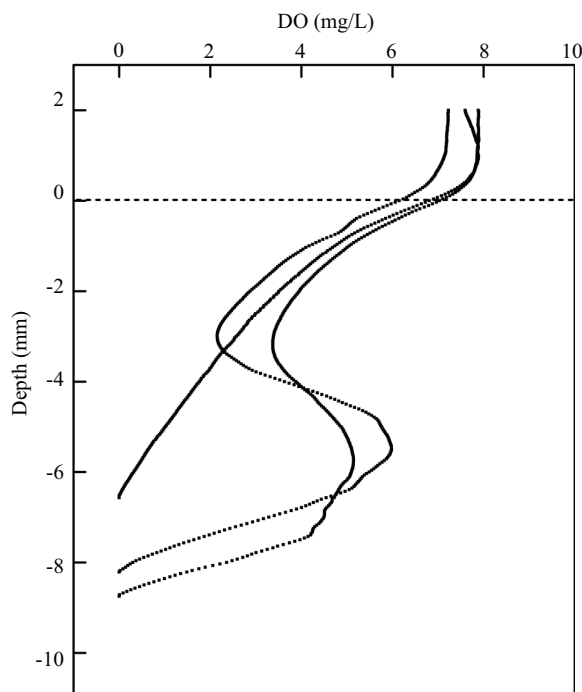
Fig. 4 Selected oxygen microprofiles in the bioturbated sediment. The horizontal dashed line at 0 mm depth indicates the SWI.
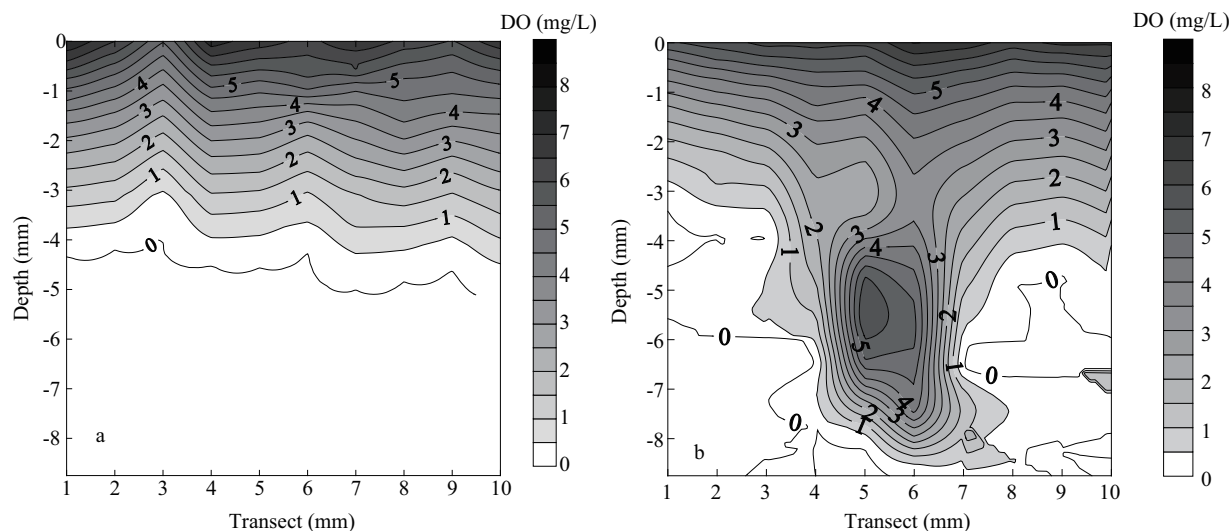
Fig. 5 Microscale distribution of oxygen on a centimeter scale in control (a) and bioturbated (b) sediments. Oxygen distribution in controls was approximately uniform. OPDs were about 4 to 5 mm in control sediments and approximately 4 to 9 mm in the sediments inhabited by chironomid larvae. Oxygen distributions in the bioturbated cores were also more complex than those in the control and showed substantial heterogeneity on a small (cm) scale.
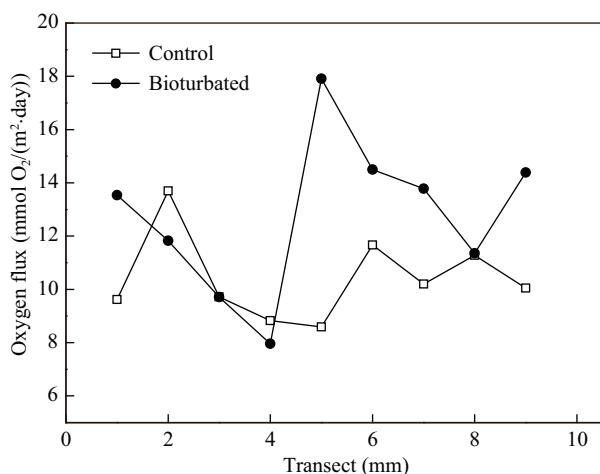

Fig. 6 Diffusive oxygen fluxes across the sediment-water interface.

2.5 mmol O_2/(m^2·day). The oxygen diffusive flux of the control and bioturbated sediments appeared to differ (T-test, $p < 0.05$).

The bioturbated cores consumed more NO_3^- and released more NH_4^+ to the water than the control (**Fig. 7**). The nitrate flux in the control was approximately -1.2 ± 1.1 mmol/(m^2·day), whereas the bioturbation of chironomids increased the NO_3^- flux to -9.1 ± 1.8 mmol/(m^2·day) (T-test, $p < 0.05$). The NH_4^+ net flux of the bioturbated sediment was found to be enhanced from 2.7 ± 0.8 to 4.5 ± 1.1 mmol/(m^2·day) ($p < 0.05$). The chironomid larvae increased the denitrification rates substantially (**Fig. 8**). The total denitrification rates in the sediment containing chironomid larvae were strongly enhanced from 0.76 ± 0.34 to 5.50 ± 1.30 mmol N/(m^2·day) ($p < 0.05$). The value of D_w increased from 0.38 ± 0.2 to 4.4 ± 1.1 mmol N/(m^2·day) ($p < 0.05$); the value of D_N increased from 0.38 ± 0.14 to 1.11 ± 0.22 mmol N/(m^2·day) ($p < 0.05$).

3 Discussion

Sediment has been shown to act as an important contributor to the nitrogen loading and eutrophication of Lake Taihu (Qin et al., 2007; Fan et al., 2004). In the present study, an increase in NH_4^+ release from the bioturbated sediment to the overlying water was also observed. This finding demonstrated that the bioturbation effects and the burrows of the chironomids intensified the role of the sediment as a source for NH_4^+ (Zhang et al., 2011; Michaud et al., 2006; Svensson, 1997). The enhancement of NH_4^+ release may be caused primarily by animal excretion and the transport of accumulated NH_4^+ in the anoxic sediment (Svensson, 1997; Emerson et al., 1984). The amount of NH_4^+ excreted by the chironomid larvae was estimated to be 0.282 mmol NH_4^+/(m^2·day) at 20°C (Fukuhara et al., 1989). The percentage of NH_4^+ excreted by the larvae, in relation to the total released amount, was calculated as 15.7%. The calculation indicates that excretion is most
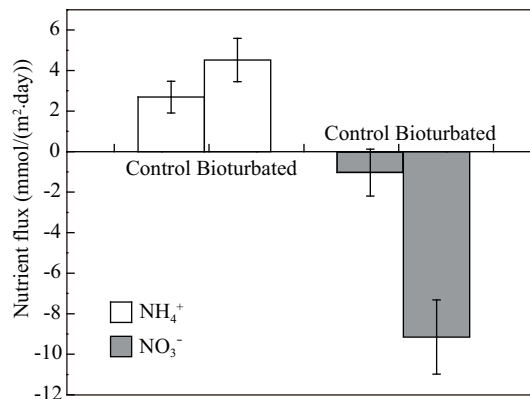
Fig. 7 Nutrient fluxes across the sediment-water interface. Nitrate was consumed from the water, and ammonium was released from the sediment. The bioturbated sediments act as net sinks for inorganic nitrogen.
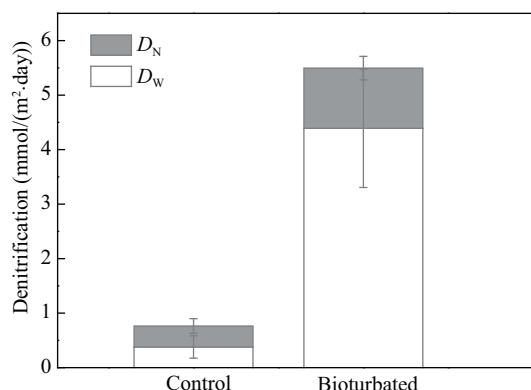
Fig. 8 Denitrification rates in the sediment. Chironomid larvae increased the denitrification rates dramatically ($p < 0.05$).

likely not the primary mechanism contributing to the overall release.

The oxygen uptake rate across the SWI is the principal parameter used to calculate the mineralization of benthic organic matter (Kristensen, 2000). The significant increase in O_2 flux produced by the chironomid larvae reflects a significant increase in the utilization of organic matter. This process would also accelerate the degradation of organic matter and enhance NH_4^+ release (Welsh, 2003). However, NH_4^+ is produced in the deep anoxic sediment and must diffuse across the oxic sediment layer before it can be released to the overlying water (Emerson et al., 1984). The oxic layer of the sediment was an effective barrier (Aller, 2001; Boudreau and Jørgensen, 2001; Jørgensen and Revsbech, 1985). Fauna activity that destroyed the original surface of the sediment and disrupted the oxic zone would accelerate NH_4^+ release to the water (Emerson et al., 1984; Fenchel, 1996; Aller, 2001; Jørgensen et al., 2005). For this reason, sediment release may be a major contributor of NH_4^+ relative to animal excretion.

Although the release of NH_4^+ from the bioturbated sediment was enhanced by a factor of 1.7, this release was exceeded by a large simultaneous increase in the NO_3^- consumption and denitrification rates (**Figs. 7** and **8**). When fauna drew oxygenated water into their burrows, NO_3^- in the overlying water was also introduced into the burrows due to the short diffusion path length for NO_3^- from the water to the anaerobic denitrification zone (Jørgensen and Revsbech, 1985; Svensson, 1997; Svensson et al., 2001). Because a prerequisite for D_W is the transport of NO_3^- from the water column to the anoxic deep sediment, more nitrate would be removed from D_W as a result of enhanced nitrate influx (Svensson 1997).

After the nitrogen contained in organic matter is remineralized through ammonification, NH_4^+ is oxidized to NO_3^- and NO_2^- via nitrification processes. This process occurs within the oxic zone, represented by a thickness of a few millimeters at the SWI and in the burrow sediments. NO_3^- or NO_2^- are then either released to the water column or retained. If retained, they would participate in reductive processes (e.g., denitrification) (Risgaard-Petersen

2004), which would increase the rate of denitrification of nitrate produced by nitrification (D_N, coupled nitrification-denitrification) (**Fig. 8**). Total denitrification rates (D_W and D_N) would also be increased under bioturbation when nitrate and ammonium flux were enhanced (Risgaard-Petersen, 2004). The difference between ammonium and nitrate flux was approximately 1.667 mmol/(m^2·day) in the control. In the bioturbated cores, the difference between nitrate and ammonium flux was –4.652 mmol/(m^2·day). The net overall effect is that the bioturbated sediments act as a net sink for inorganic nitrogen. This finding corresponds well with the results of Svensson (1997).

Complex and three-dimensional burrows play an important role in these source-sink transition processes. Our visual observations of light-colored oxidized burrow walls demonstrated that the larvae introduced oxygen into the deep sediment. The 2-D oxygen distribution also showed that the oxygen concentration in the burrows was higher than in the adjacent sediment. These complex burrows would become "hot spots", as described by Glud (2009). We did not calculate the volume of the increased oxic zone (hot spots) in the present study. However, according to Jørgensen (2005), burrows extending down into the anoxic sediment increased the oxic sediment volume by 2% to 10% in Arctic fjord sediments (Svalbard, Barents Sea). It is hypothesized that the physical mixing of sediment during burrow building and maintenance activities can serve to enhance the microbial communities in the sediment. The unique physical and chemical characteristics of these burrows (Lovelll, 1996; Stief and de Beer, 2002) are associated with strong influences on benthic carbon mineralization (Kristensen, 2000) and the nitrogen cycle (denitrification, nitrification and nitrogen fixed processes) (Nielsen et al., 2004; Risgaard-Petersen, 2004; Stief and de Beer, 2002; Welsh, 2003).

Diverse studies have shown that the availability of nitrogen is one of the key factors responsible for eutrophication and algal blooms (Xu et al., 2010; McCarthy et al., 2007; Qin et al., 2007). Under such conditions, the direct and indirect bioturbation effects produced by *Tanypus chinensis* larvae are expected to promote the loss of nitrogen and would be helpful in removing nitrogen loads and controlling harmful algal blooms in Lake Taihu. However, our results are based on microcosm simulation experiments. For this reason, it is not yet possible to evaluate the decrease in the amount of nitrogen in the water in Lake Taihu resulting from bioturbation effects. Thus, *in situ* and larger-scale studies should be the focus of future work.

4 Conclusions

This study confirmed the ecological importance of *Tanypus chinensis* larvae and showed that the bioturbating activities of these larvae can potentially affect the nitrogen cycle.

The bioturbated sediments acted as a net sink for inorganic nitrogen. Although the ammonium release from sediment to water was increased, the nitrate flux from water to sediment and the potential for sediment denitrification was also increased. The amount of inorganic nitrogen removed from the water body was greater than the amount released from the sediment. Thus, direct and indirect bioturbation effects cause the bioturbated sediment to act as a net sink for inorganic nitrogen. This process is expected to aid in removing nitrogen loads in Lake Taihu.

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