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Bioturbation Effects of *Chironomid* Larvae on Nitrogen Release and Ammonia-Oxidizing Bacteria Abundance in Sediments

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Abstract: The purpose of this work was to reveal the *Chironomid* larvae bioturbation impact on N release and to find the mechanism of bioturbation to N conversion at the SWI (sediment–water interface). Sampling at four points during a 35-day incubation experiment was conducted. Two in situ techniques (microelectrode and Peeper) were used to capture more realistic and accurate microenvironment information around U-shaped corridors. The results demonstrate that the concentrations of ammonia nitrogen (NH_4^+) and nitrate nitrogen (NO_3^-) decreased by 21.26% and 19.50% in sediment and increased by 8.65% and 49.82% in the overlying water compared to the control treatment, respectively. An inverse relationship was observed between NH_4^+ and NO_3^- concentrations in pore water in *Chironomid* larvae treatment, and they were significantly negatively/positively correlated with AOB (ammonia-oxidizing bacteria) abundance, respectively. This study confirmed that the *Chironomid* larvae bioturbation promoted the N (NH_4^+ and NO_3^-) release from sediment by in situ techniques, and a part of NH_4^+ converted into NO_3^- during their flow into the overlying water through the nitrification affected by AOB. Furthermore, the main depth of bioturbation influence is approximately 12 cm below the SWI and the most significant bioturbation effect was observed from days 15 to 25.

Keywords: *Chironomid* larvae; bioturbation; ammonia nitrogen; nitrate nitrogen; ammonia-oxidizing bacteria

1. Introduction

Nitrogen (N) is an essential nutrient for aquatic plants and algae in freshwater ecosystems, and its significant role in water eutrophication has also attracted great attention over the last several decades [1]. Some of the N taken up by aquatic plants can result from the ammonia nitrogen (NH_4^+) release of the sediment, which is also called an internal load of N Sediment can contribute a substantial amount of N to the water column and support algal blooms, particularly after a reduction of the external N input in lakes [2]. There is a range of environmental factors influencing the release of N, such as temperature, pH, Eh, and faunal hydro dynamics [3–5]. However, a significant regulating factor—bioturbation—is almost neglected in freshwater.

According to the research of Lewandowski and Hupfer (2005) [6], bioturbation in aquatic environment is generated by the activity of animals within the upper sediment layers. The activity can alter the structure and properties of the sediment, which further influences the release of N from sediments and the flux of N to the overlying water [7–9]. The bioturbation of benthic animals changes dissolved oxygen (DO), organic matter, redox conditions and microbial activity at the SWI [10,11]. Hence, these changes likely influence N content and distribution in sediments and make the migration and conversion of N species more complicated in sediments. As a typical benthonic animal, *Chironomid* larvae have received extensive attention and research. *Chironomid* larvae can enhance the outflow of NH_4^+ from the sediment to the overlying water across the sediment–water interface [12–14]. While others show the opposite effect, *Chironomid* larvae increased the nitrate nitrogen (NO_3^-) release rates [15]. Moreover, exchange of inorganic nitrogen was slightly influenced by added *Chironomid* larvae [16]. Nitrification and denitrification are important processes of N species conversion. In these studies, the results indicate that bioturbation can have a great impact on denitrification, and very low nitrification can occur [15,17]. However, another study shows bioturbation related to ammonia oxidation, which is generally considered to be the critical rate-limiting step of the nitrification process [18]. Since different phenomena behind the larvae bioturbation on N release have been observed, the apparently contradictory results may illustrate the relative importance of capturing more realistic and accurate information about the sediment microenvironment.

Study of the N release process in sediments should be conducted at the real environmental status holding constant to monitor the spatial variations of N and the influencing factors (especially DO and Eh). However, in order to obtain the relevant information, previous studies have often used the invasive ex-situ sampling technique. This method will destroy the structure of the sediment layer, inevitably exposing the sediments to the air and will easily change the original properties of the samples, which results in a considerable inaccuracy of the N content [7]. Peeper technology has provided promising alternatives to overcome this shortage with a slight disturbance of the sediment [19]. It is an in situ technique able enough to satisfy the requirement of studying the *Chironomid* larvae bioturbation effects and can obtain more accurate and authentic N information from the sediment. In order to determine the environmental conditions around U-shaped corridors, the microelectrode measurement system was introduced, in which a microelectrode with a tip diameter can detect the micro-interfacial environment in a nondestructive or quasi-nondestructive manner [20,21]. The application of a microelectrode thus improves the accuracy of our study.

This study aimed to investigate the effect of *Chironomid* larvae bioturbation on N release and conversion at the SWI. Two in-situ technologies were used in order to obtain more accurate and true information about the sediment's micro environment. The microelectrode measurement system was used to detect Eh and O_2 in sediments, and the Peeper technology was used to collect pore water for NH_4^+ and NO_3^- detection. Then, the concentration of NH_4^+, NO_3^- and the abundance of ammonia-oxidizing bacteria (AOB) in sediments were analyzed at 1.5 cm intervals. All of the data assists in advancing the understanding of N release and conversion under the bioturbation in eutrophic lake sediments.

2. Materials and Methods

2.1. Experimental Microcosm Set up

Sediment used in the experiment was collected from the eutrophic Lake Dazong (31°30′31.1″ N, 120°10′31.0″ E) in the lower basin of the Huai River during November 2016. Twelve sediment cores (11 cm in diameter, 30 cm in length) were taken by a gravity corer (11 cm × 50 cm, Rigo Co., Chiba, Japan). Meanwhile, overlying water was collected with plastic vessels for simulation experiments in the laboratory. At the same sampling occasion, the *Chironomid* larvae were collected. All of the sediment cores were sectioned at a 1.5 cm interval with the same depth then pooled together, sieved with a 0.6 mm pore-size mesh, and thoroughly homogenized. Finally, the sediments were put

into eight Plexiglas tubes at their original depth. Afterwards, every fourth sediment core was put into a tank with the addition of 40 cm of filtered lake water. The fourth instar *Chironomid* larvae were divided from the sediment, 72 larvae were equally added into four sediment cores approaching the population density in the sampling site (1887 ind./m^2), while the remaining four cores were kept as controls without the addition of *Chironomid* larvae. Single *Chironomid* larvae that were found dead were carefully removed and were replaced by individuals of approximately the same size. *Chironomid* larvae were precultured for 15 days. The temperature of the water in the tank was 15 °C, controlled by the circulating water system. The water was pumped with air for 10 min per hour to maintain the oxic environment.

2.2. Preparation of Peeper and Sampling

The Peeper technique was used to collect pore water for NH_4^+ and NO_3^- detection. The principle of this technique is to use a dialysis membrane to separate multi-chambered receiver solutions from the surrounding pore water. The first dialysis pore water device, known as a Peeper, was developed by Hesslein (1976) [22] and Mayer (1976) [23]. The Peeper probes were prepared according to Xu et al. (2012) [19], and they have 75 chambers (18 mm × 1.0 mm × 1.0 mm, length × width × height) on a base plate. Each pair of adjacent chambers was separated horizontally by a 1 mm thick wall, producing a vertical resolution of 2.0 mm for sampling. The chambers were filled with deionized water and covered by a 0.10 mm PVDF membrane (Durapore®, 0.45 μm pore size, Millipore, Burlington, MA, USA) to separate the inner chamber from the surrounding pore water. All the Peeper probes were soaked in purified water and deoxygenated with N_2 for more than 16 h before they were put into the sediment cores. The pore water was collected by the Peeper probes which had been deployed in sediments for 48 h. After removal from the sediments, the Peeper probes were immediately cleaned sequentially by wet filter papers and deionzied water. The water samples were collected by the transferpettor from the chamber in the Peeper probes and then analyzed as soon as possible.

On the 5th day, after the retrieval of Peepers probes, the sediment cores were obtained from the control tank and the *Chironomid* larvae treatment tank, respectively. The sediments were sliced into 10 sections in 1.5 cm intervals, and then the contents NH_4^+, NO_3^- and the abundance of AOB in sediments were measured. Then, the 5th day's procedure were repeated three times on the 15th, 25th and 35th days, respectively.

2.3. Analytical Methods

Water samples were kept in a cooler at 4 °C before analysis. The DO concentration and Eh were determined by a microelectrode system (OX 100 and Redox 100, Unisense, Aarhus, Denmark). The concentrations of NH_4^+ and NO_3^- were detected by a multimode reader (M2e, Molecular Devices, San Jose, CA, USA) and a flow injection analyzer (Skalar SAN++, SKALAR, Breda, The Netherlands), respectively. The NH_4^+ and NO_3^- in sediments were extracted with saturated KCl solution before detection. All analytical operations were conducted using of strict quality control guide lines and analysis of replicates. All samples were measured three times and the mean of the results were taken to eradicate any discrepancies. The basic sediment characteristics are shown in Table 1.

Table 1. Characteristics of sediment used in the simulation experiment.

Sediment Layer (cm)	Cay (%)	Silt (%)	Sand (%)	NO_3^- (mg·kg^{-1})	NH_4^+ (mg·kg^{-1})	TN (mg·kg^{-1})
0–4	2.69	43.12	54.19	12.92	67.18	2412.66
4–8	3.04	42.60	54.36	6.32	99	2074.7
8–12	3.39	44.34	52.26	6.52	128.44	1823.36
12–16	6.37	49.68	43.96	7.37	152.07	1694.65

The DNA was extracted from a 0.5 g freeze-dried and sieved sediment sample using an extraction and purification agent (FastDNA® Spin Kit for Soil, MP Biomedical, Santa Ana, CA, USA). The samples

were washed twice with precooled 70% alcohol, then were suspended in a sterilized Tris-EDTA (TE) buffer solution. The final volume of the sample was 50 μL. DNA was analyzed by agarose electrophoresis and the samples were stored below −20 °C. The DNA was extracted three times from each sample before consolidation to ensure the uniformity of the microbes. The consolidated DNA was diluted 10-fold and then used as a template. The AOB gene copies were quantified using primers amoA-1F (5′-GGGGTTTCTACTGGTGGT-3′) and amoA-2R (5′-CCCCTCKGSAAAGCCTTCTTC-3′) in a 25-μL amplification system (Corbett, RG65H0, Sydney, Australia) [24]. The PCR product was analyzed by gel electrophoresis with a 1.0% agarose gel. The sample was treated with fluorescent dye (0.5 μg/mL) before a photo was taken with a gel imager (Bio-Rad, Chemi Doc XRS Total, Hercules, CA, USA).

3. Results

3.1. Changes of DO and Eh in Sediment Cores

The distribution of DO in sediment cores measured at a 100 μm vertical resolution are shown in Figure 1. With the increase of the sediment depth, the content of DO decreased both the control treatment (i.e, non-bioturbated cores) and the *Chironomid* larvae treatment (i.e., bioturbated cores) at the four sampling times. *Chironomid* larvae bioturbation has little effect on the content of DO in the overlying water but has a great influence on the content of DO in sediment, and significantly increased the penetration depths of oxygen (OPD) (one-way ANOVA, treatment effect, $p < 0.05$), especially from 15th day to 25th day. From beginning to end, the OPD in the control were about 2.0 mm. However, the OPD values were 4.0, 6.5, 8.2 and 5.0 mm in the presence of *Chironomid* larvae, which were 2.0, 3.3, 4.1 and 2.5 times the size of the control, respectively. The result is similar to the report by Chen et al. (2015) [25], who has investigated the effects of *Chironomid* larvae bioturbation on the lability of phosphorus (P) in sediments. *Chironomid* larvae can bioirrigated oxygen-rich waters into their galleries and increased the penetration depth of oxygen [6,26], and the reason they divert overlying water into corridors is for food and oxygen (O_2) [27].

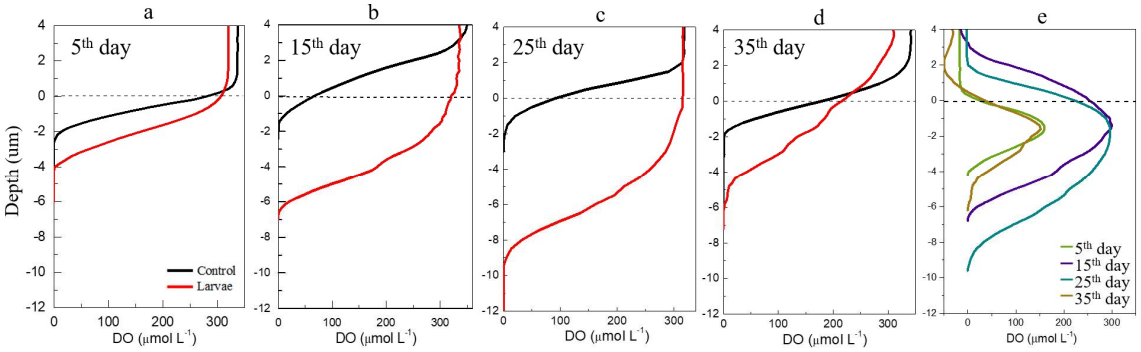

Figure 1. DO profiles in sediment cores of the control and the *Chironomid* larvae treatment. The horizontal dashed line at zero indicates the sediment–water interface (SWI); over the line indicates overlying water; under the line indicates sediments. (e) DO changes in treatment minus changes in control on the 5th (**a**), 15th (**b**), 25th (**c**) and 35th (**d**) day, respectively.

The distribution of Eh values in sediment cores measured at a 100 μm vertical resolution are shown in Figure 2. The *Chironomid* larvae bioturbation significantly increased Eh values in sediment (one-way ANOVA, treatment effect, $p < 0.05$), which were consistent with the changes of DO content. The average values of Eh were 282.61, 282.07, 290.45 and 296.74 mv on the 5th, 15th, 25th and 35th day in the control treatment, respectively. The average values were 344.78, 324.38, 368.87 and 338.28 mv in the presence of *Chironomid* larvae, with an increase by 22%, 15%, 27% and 14%, respectively. The effects of *Chironomid* larvae bioturbation on redox state (Eh) were similar to those on DO. It can be explained

that the larvae continuously bioirrigated oxygen-rich water into their galleries and increased Eh values in the sediments.

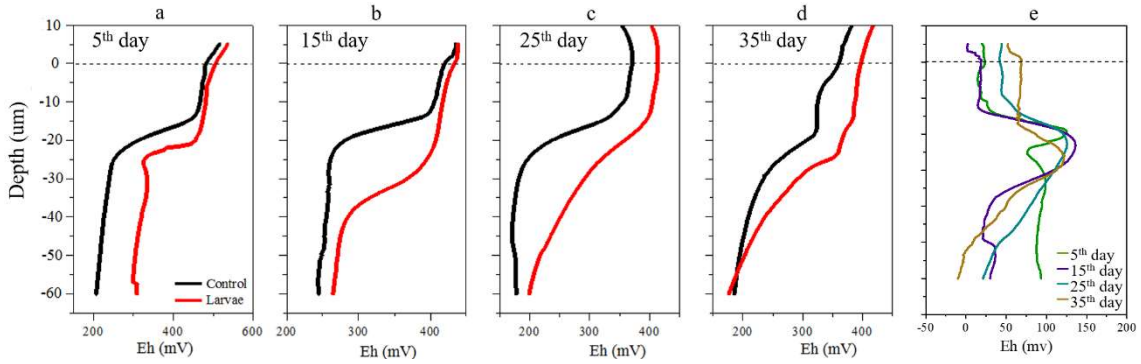

Figure 2. Eh profiles in sediment cores of the control and the *Chironomid* larvae treatment. The horizontal dashed line at zero indicates the sediment–water interface (SWI); over the line indicates overlying water; under the line indicates sediments. (e) Eh changes in treatment minus changes in control on the 5th (**a**), 15th (**b**), 25th (**c**) and 35th (**d**) day, respectively.

3.2. Changes of NH_4^+ and NO_3^- in Overlying Water

The NH_4^+ concentrations in overlying water are shown in Figure 3a. The NH_4^+ concentrations of the control treatment were increased slowly during the whole experimental process, and the values were about 1.50, 1.62 1.74 and 1.85 mg·L^{-1}, respectively. The introduction of *Chironomid* larvae has a strong effect on NH_4^+ concentrations in the overlying water at four stages. The values of NH_4^+ concentrations were decreased on the first two stages and were increased on the last two stages in the larvae treatment, and the values were about 1.37, 1.22, 1.86 and 2.01 mg·L^{-1}, respectively. The smallest concentration appeared on the 15th day, with a decrease by 24.69% compared with the control treatment, followed by an increasing trend with incubation time, and the NH_4^+ concentrations increased by 8.65% relative to the control treatment at the end of the experiment. The maximum variation between the *Chironomid* larvae treatment was observed from day 15 to 25 (Tukey's HSD test, $p < 0.001$).

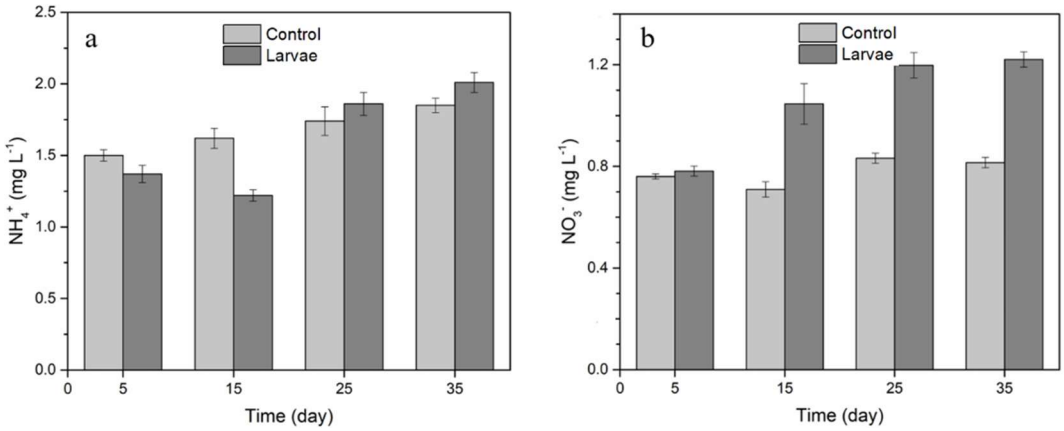

Figure 3. Ammonium nitrogen (NH_4^+) and nitrate nitrogen (NO_3^-) profiles in the overlying water of the control and the *Chironomid* larvae treatment. (**a**) NH_4^+ and (**b**) NO_3^-.

The NO_3^- concentrations in overlying water are shown in Figure 3b. The NO_3^- contents in the overlying water were about 0.7 ± 0.1 mg·L^{-1} in the control group during the whole experiment process. In the larvae treatment, the values were about 0.78, 1.05, 1.20 and 1.22 mg·L^{-1}, with an increase by 2.8%, 47.5%, 44.0% and 49.8%, respectively. The bioturbation of *Chironomid* larvae continuously increased

NO_3^- concentrations in the overlying water during the whole experimental period, and especially on the 15th day, it showed the most obvious increases (Tukey's HSD test, $p < 0.024$).

3.3. Changes of NH_4^+ and NO_3^- in Sediments

The distribution of NH_4^+ and NO_3^- in sediments are shown in Figure 4. During the whole experiment process, NH_4^+ and NO_3^- concentrations of the larvae treatment was lower than that of the control treatment. The values of NH_4^+ decreased by 3.46%, 13.12%, 18.68% and 21.26% relative to the control treatment, respectively. The values of NO_3^- decreased by 9.92%, 18.35%, 18.55% and 19.50% relative to the control treatment, respectively. The largest difference of NH_4^+ and NO_3^- contents with or without bioturbation occurred on the 35th day (Tukey's HSD test, $p < 0.000$). It was shown that the difference of NH_4^+ and NO_3^- between the larvae treatment and control treatment is appeared from 0 to 15 cm (the maximum detection depth), and the main influence depth is approximately 12 cm below the SWI. The *Chironomid* larvae bioturbation was helpful to the decrease of NH_4^+ and NO_3^- in sediments. In other words, it promotes the release of sediment N. The decrease of N content in sediments is mainly due to building caves behavior of the *Chironomid* larvae increase the surface area of SWI, and the biological diversion increased NH_4^+ and NO_3^- diffuse into the pore water.

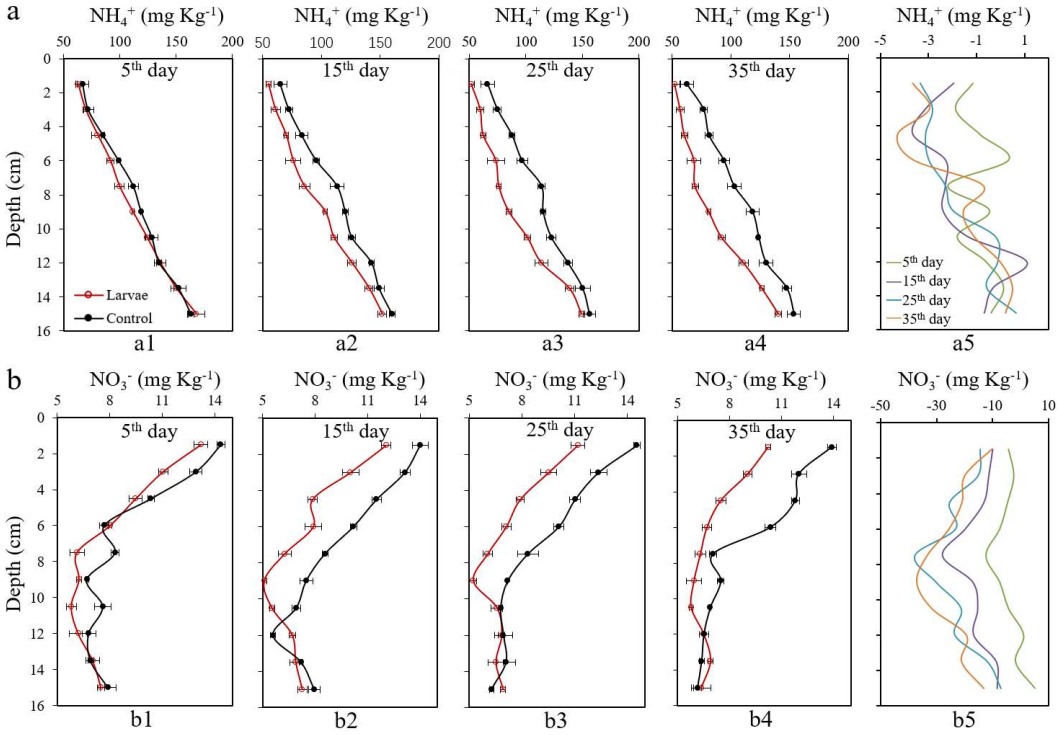

Figure 4. Ammonium nitrogen (NH_4^+) and nitrate nitrogen (NO_3^-) profiles in sediments of the control and the *Chironomid* larvae treatment. (**a**) NH_4^+ and (**b**) NO_3^-. (**a5**) NH_4^+ and (**b5**) NO_3^- in sediments changes in treatment minus changes in control on the 5th, 15th, 25th and 35th day, respectively.

3.4. Changes of NH_4^+ and NO_3^- in Pore Water

The ammonium nitrogen (NH_4^+) profiles in pore water collected by Peeper are shown in Figure 5a. The concentrations of NH_4^+ in the larvae treatment were lower than those in the control treatment for the duration of the experiment. In other words, the bioturbation of *Chironomid* larvae reduced the concentration of NH_4^+ in pore water, and the depth of bioturbation influence was up to 15 cm. At the set temperature (15 °C), as time went on, concentration differences of NH_4^+ with or without bioturbation were firstly increased and then decreased. This shows that the bioturbation effect on NH4$^+$ first increases and then weakens. The bioturbation effects lasted more than 35 days, and the

strongest bioturbation effect was observed on the 15th day (Tukey's HSD test, $p < 0.000$). On that day, NH_4^+ in the larvae treatment and the control treatment had the most obvious difference at the same layer. On the 25th and 35th day, the differences of NH_4^+ in the two treatments were gradually reduced.

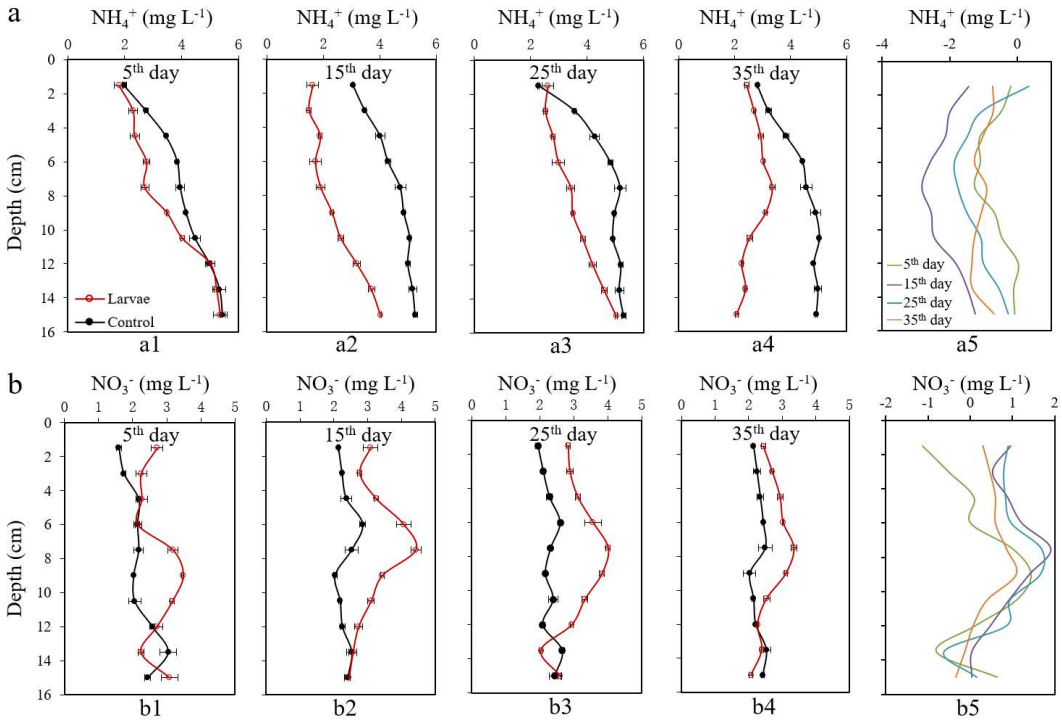

Figure 5. Ammonium nitrogen (NH_4^+) and nitrate nitrogen (NO_3^-) profiles in pore water collected by Peeper of the control and the *Chironomid* larvae treatment. (**a**) NH_4^+ and (**b**) NO_3^-. (**a5**) NH_4^+ and (**b5**) NO_3^- in pore water changes in treatment minus changes in control on the 5th, 15th, 25th and 35th day, respectively.

The nitrate nitrogen (NO_3^-) profiles in pore water collected by Peeper are shown in Figure 5b. On the whole, the larvae bioturbation increased the concentration of nitrate nitrogen (NO_3^-). The depth of bioturbation influence is affected the whole 15 cm of the microcosm, and especially above 12 cm. Like NH_4^+, concentration differences of NO_3^- with or without bioturbation were firstly increased and then decreased. This shows that the bioturbation effect on NO_3^- first increases and then weakens. The bioturbation effects lasted more than 35 days, and the strongest bioturbation effect was observed on the 25th day (Tukey's HSD test, $p < 0.002$). The maximal NO_3^- concentration value in the larvae treatment was up to 4.42 mg·L^{-1} on the 15th day. Then, on the 35th day, the differences of NO_3^- in the two treatments were gradually reduced.

3.5. Changes of Ammonia-Oxidizing Bacteria in Sediments

Ammonia-oxidizing bacteria (AOB) are important nitrifying bacteria, the distribution of which in the sediments was studied in this research. The abundance of AOB in sediments of control and larvae treatment are shown on Figure 6. The average AOB abundance values of larvae bioturbation were larger than those of the control at four stages. Obviously, there are significant differences between larvae treatment and control treatment. The AOB abundance of control treatment was little changed in the whole experimental process. In the early stage of the experiment, the AOB abundance of *Chironomid* larvae treatment increased quickly and reached its highest value at 7.16 × 107 copies/(g dw) on the 15th day, and then it decreased on the 25th day and the 35th day. This shows that the bioturbation effect on AOB abundance increases first and then weakens. The greatest difference of AOB between

the *Chironomid* larvae sediments and the control sediments was observed on the 15th day (Tukey's HSD test, $p < 0.020$).

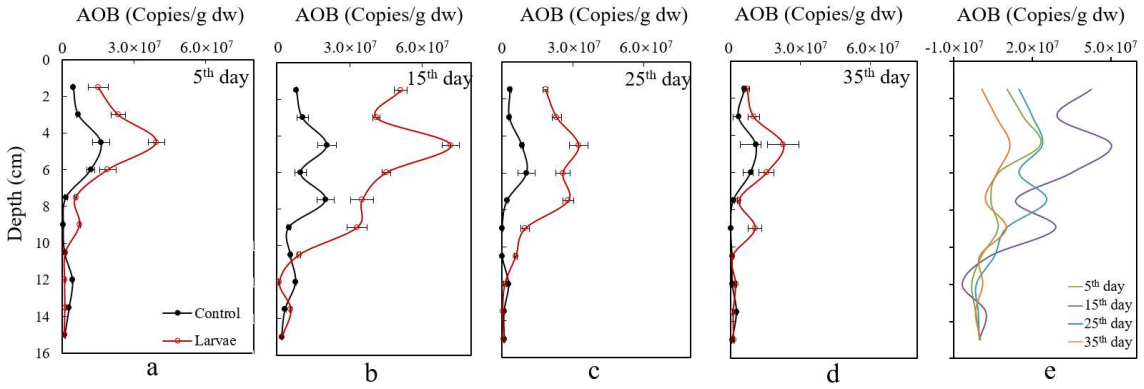

Figure 6. Ammonia-oxidizing bacteria (AOB) profiles in sediments of the control and the *Chironomid* larvae treatment. Dw: dry sediment. (e) AOB abundance changes in treatment minus changes in control on the 5th (**a**), 15th (**b**), 25th (**c**) and 35th (**d**) day, respectively.

4. Discussion

4.1. Assessment of the Effects of the Larvae Bioturbation on Sediment N

This study has revealed considerable changes of NH_4^+ and NO_3^- at the SWI with bioturbation in *Chironomid* larvae treatment and without bioturbation in control treatment, respectively. In-situ techniques used in this study could detect well the effects of larvae bioturbation. The results indicate that bioturbation by *Chironomid* larvae has a major impact on N release, reflected by the large decreases of NH_4^+ and NO_3^- in the sediment (Figure 4), respectively. The mean concentrations of NH_4^+ and NO_3^- in the bioturbation treatment were reduced by 21.26% and 19.50% compared to the control treatment, respectively. The phenomenon was also reported by Lewandowski et al. (2007) [28], and they found that the NH_4^+ content was reduced by 25% with C. plumosus bioturbation. Except for affecting N release, the bioturbation by *Chironomid* larvae can also result in N conversion, which can be reflected by the large increase of NO_3^- concentrations in the overlying water and pore water, but NH_4^+ concentrations decreased in pore water and only slightly increased in the overlying water. An inverse relationship was observed between NH_4^+ and NO_3^- concentrations in pore water in *Chironomid* larvae treatment. There is no relationship in control treatment but showed a significant negative correlation in *Chironomid* larvae treatment (Table 2), especially on the 15th day.

Table 2. Correlation analyses between NH_4^+ and NO_3^- profiles in pore water of the control and the *Chironomid* larvae treatment ($p = 0.05$).

Treatment	Time (day)	r
Chironomid larvae	5	0.477
	15	−0.612 **
	25	−0.411 *
	35	−0.570 *
Control	5	0.181
	15	0.163
	25	0.233
	35	0.199

Note: **: significant correlation at $p = 0.01$, *: significant correlation at $p = 0.05$.

The influence of larvae bioturbation on N release was mainly from days 15 to 25. In this stage, some extreme values appeared, including the minimum concentration of NH_4^+ and the maximum concentration of NO_3^- in overlying water, the most obvious concentration difference of NH_4^+/NO_3^- in pore water and the highest abundance of AOB. These phenomena may imply that AOB plays a significant role in the release of nitrogen from sediment. Then, we analyzed the correlation of NH_4^+ vs. AOB and NO_3^- vs. AOB profiles in pore water of the *Chironomid* larvae treatment (Table 3). The results showed that there was a significant negative correlation between NH_4^+ and AOB, and a significant positive correlation between NO_3^- and AOB. Especially on the 15th and 25th day, the correlation was the most significant, and the values were −0.838 ** and 0.785 **, −0.843 ** and 0.711 *, respectively. Therefore, we infer that nitrification process mediated by AOB plays an important role in N release and conversion in sediments, in which NH_4^+ was converted into NO_3^-.

Table 3. Correlation analyses NH_4^+ vs. AOB and NO_3^- vs. AOB profiles in pore water of the *Chironomid* larvae treatment (p = 0.05).

Items	Time (day)	r
NH_4^+ vs. AOB	5	−0.731 *
	15	−0.838 **
	25	−0.843 **
	35	−0.497
NO_3^- vs. AOB	5	0.655
	15	0.785 **
	25	0.711 *
	35	0.632

Note: **: significant correlation at p = 0.01, *: significant correlation at p = 0.05.

Furthermore, the depths of bioturbation effect on NH_4^+ and NO_3^- concentrations in pore water and sediments affected the whole 15 cm of the microcosm: Especially above 12 cm, which was shorter than the 18 cm according to Caffrey [29] and deeper than the 7 cm according to Chen [25] and 100 mm according to Lewandowski [28]. Thus, this proved that the application of these in situ technologies is reliable, and the main bioturbation influence depth for N release was about 12 cm below the SWI.

4.2. Bioturbation Mechanism on Sediment N

Chironomid larvae play an important role in the lake sediment environment via biological diversion, cave building, absorption, digestion, defecation and secretion [6,26]. The action of diversion and the behavior of building caves caused dissolved substances in pore water transport to the overlying water easily and efficiently [30]. Haruo Fukuhara [12] found that the larvae of *Chironomus plumosus* caused an enhancement of inorganic nitrogen release (mainly NH_4^+). This finding is inconsistent with the result of our study. We found that *Chironomid* larvae did increase the N release (specifically, increasing NO_3^- release). This difference may be due to the reasons outlined below.

Chironomid larvae not only increase the surface area of SWI, but their burrows also become sites of high bacterial numbers and high metabolic activity compared to the surrounding sediment. *Chironomid* larvae bio-irrigated oxygen-rich overlying water into their U-shaped corridors, resulting in an increase in DO concentrations (Figure 1) in sediments. The imported oxygen diffused through the burrow walls into the surrounding sediments, leading to redox zones concentrically distributed around the tubes (Figure 2), which provided AOB with proliferation, activity sites, and enhanced the activity of AOB (Figure 6) [31,32]. Combined with the NH_4^+ (released from the sediment) and O_2 (bio-irrigated from the overlying water), AOB promoted the nitration reaction in the cave and the channel wall, which facilitated the conversion of NH_4^+ in pore water into NO_3^- (Figure 7) [33]. A part of NH_4^+ released from the sediment flowed into the overlying water via molecular diffusion and another part was converted into NO_3^- through the nitrification affected by AOB. Hence, NH_4^+ (Figures 4a and 5a)

was decreased in the pore water and sediment, and the NO_3^- (Figures 3b and 5b) in the overlying water and pore water was increased. The studies indicate that the O_2 and AOB play important roles in controlling the release and conversion of N in sediments, i.e., NH_4^+ is converted to NO_3^- in oxidized sediments, which is consistent with previous studies [34,35].

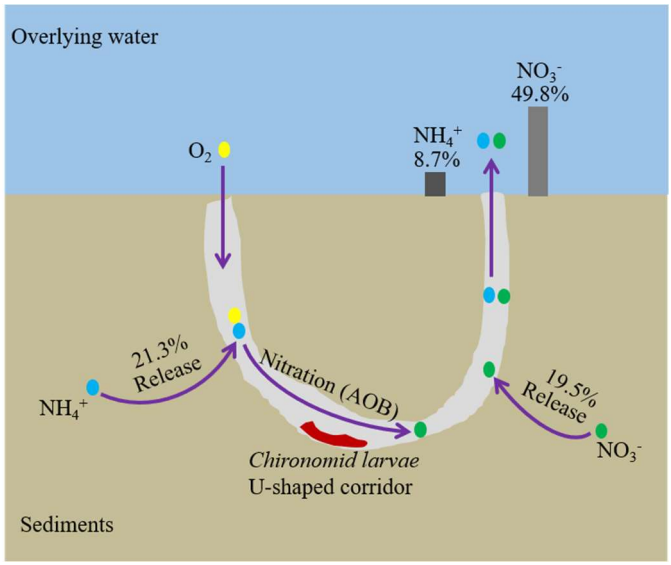

Figure 7. Schematic illustration of the processes of *Chironomid* larvae bioturbation impact on N release and conversion at the SWI.

5. Conclusions

Through the use of two in situ technologies, we clearly found that the bioturbation of *Chironomid* larvae facilitated the release and conversion of N in sediments. The concentrations of NH_4^+ and NO_3^- decreased by 21.26% and 19.50% in sediment, and increased by 8.65% and 49.82% in overlying water compared to the control treatment, respectively. The bioturbation of *Chironomid* larvae increased the release of NH_4^+ and NO_3^- from sediments to the overlying water. The bioturbation also enhanced the nitration reaction, and a part of NH_4^+ released from the sediment to the overlying was converted into NO_3^- by AOB with adequate DO. Furthermore, the main influence depths of *Chironomid* larvae bioturbation were approximately 12 cm below the SWI, and the most significant bioturbation effect was observed from days 15 to 25. The research results gained by in situ technologies can provide a more comprehensive and accurate understanding of the effect of bioturbation on N release at the SWI and also contribute to water eutrophication management.

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References

1. Hou, D.; He, J.; Lü, C.; Dong, S.; Wang, J.; Xie, Z.; Zhang, F. Spatial variations and distributions of phosphorus and nitrogen in bottom sediments from a typical north-temperate lake, China. *Environ. Earth Sci.* **2014**, *71*, 3063–3079. [CrossRef]

2. Qin, B.; Zhu, G.; Lu, Z.; Luo, L.; Gao, G.; Gu, B. Estimation of internal nutrient release in large shallow Lake Taihu, China. *Sci. China Earth Sci.* **2006**, *49*, 38–50. [CrossRef]
3. Qin, B.; Weiping, H.U.; Gao, G. Dynamics of sediment resus-pension and the conceptual schema of nutrient release in the large shallow Lake Taihu, China. *Chin. Sci. Bull.* **2004**, *49*, 54–64. [CrossRef]
4. Danielsson, A.; Jönsson, A.; Rahm, L. Resuspension patterns in the Baltic proper. *J. Sea Res.* **2007**, *57*, 257–269. [CrossRef]
5. Spears, B.M.; Carvalho, L.; Perkins, R.; Paterson, D.M. Effects of light on sediment nutrient flux and water column nutrient stoichiometry in a shallow lake. *Water Res.* **2008**, *42*, 977. [CrossRef] [PubMed]
6. Lewandowski, J.; Hupfer, M. Effect of Macrozoobenthos on Two-Dimensional Small-Scale Heterogeneity of Pore Water Phosphorus Concentrations in Lake Sediments: A Laboratory Study. *Limnol. Oceanogr.* **2005**, *50*, 1106–1118. [CrossRef]
7. Hansen, K.; Mouridsen, S.; Kristensen, E. The impact of *Chironomus plumosus* larvae on organic matter decay and nutrient (N, P) exchange in a shallow eutrophic lake sediment following a phytoplankton sedimentation. *Hydrobiologia* **1997**, *364*, 65–74. [CrossRef]
8. Janssen, F.; Huettel, M.; Witte, U. Pore-water advection and solute fluxes in permeable marine sediments (II): Benthic respiration at three sandy sites with different permeabilities (German Bight, North Sea). *Limnol. Oceanogr.* **2005**, *50*, 779–792. [CrossRef]
9. Meysman, F.J.R.; Galaktionov, O.S.; Britta, G.; Middelburg, J.J. Bioirrigation in permeable sediments: Advective pore water transport induced by burrow ventilation. *Limnol. Oceanogr.* **2006**, *51*, 142–156. [CrossRef]
10. Zhang, L.; Liao, Q.; Gu, X.; He, W.; Zhang, Z.; Fan, C. Oxygen and phosphorus dynamics in freshwater sediment after the deposition of flocculated cyanobacteria and the role of tubificid worms. *J. Hazard. Mater.* **2014**, *266*, 1–9. [CrossRef] [PubMed]
11. Ding, S.; Han, C.; Wang, Y.; Yao, L.; Wang, Y.; Xu, D.; Williams, P.N.; Zhang, C. In situ, high-resolution imaging of labile phosphorus in sediments of a large eutrophic lake. *Water Res.* **2015**, *74*, 100–109. [CrossRef] [PubMed]
12. Fukuhara, H.; Sakamoto, M. Enhancement of Inorganic Nitrogen and Phosphate Release from Lake Sediment by Tubificid Worms and Chironomid Larvae. *Oikos* **1987**, *48*, 312–320. [CrossRef]
13. Andersen, F.; Jørgensen, M.; Jensen, H.S. The Influence of *Chironomus plumosus* Larvae on Nutrient Fluxes and Phosphorus Fractions in Aluminum Treated Lake Sediment. *Water, Air, Soil Pollut.* **2006**, *6*, 101–110. [CrossRef]
14. Reitzel, K.; Lotter, S.; Dubke, M.; Egemose, S.; Jensen, H.S.; Andersen, F.Ø. Effects of Phoslock ®treatment and chironomids on the exchange of nutrients between sediment and water. *Hydrobiologia* **2013**, *703*, 189–202. [CrossRef]
15. Tátrai, I. Experiments on Nitrogen and Phosphorus Release by Chironomus ex gr. *plumosus* from the Sediments of Lake Balaton, Hungary. *Int. Rev. Hydrobiol.* **2010**, *73*, 627–640.
16. Granéli, W. The influence of *Chironomus plumosus* larvae on the exchange of dissolved substances between sediment and water. *Hydrobiologia* **1979**, *66*, 149–159. [CrossRef]
17. Svensson, J.; Leonardson, L. Effects of bioturbation by tube-dwelling chironomid larvae on oxygen uptake and denitrification in eutrophic lake sediments. *Plant J.* **2010**, *35*, 289–300. [CrossRef]
18. Laverock, B.; Kitidis, V.; Tait, K.; Gilbert, J.A.; Osborn, A.M.; Widdicombe, S. Bioturbation determines the response of benthic ammonia-oxidizing microorganisms to ocean acidification. *Philos. Trans. R. Soc. Lond.* **2013**, *368*, 20120441. [CrossRef] [PubMed]
19. Xu, D.; Wu, W.; Ding, S.; Sun, Q.; Zhang, C. A high-resolution dialysis technique for rapid determination of dissolved reactive phosphate and ferrous iron in pore water of sediments. *Sci. Total Environ.* **2012**, *422*, 245–252. [CrossRef] [PubMed]
20. Revsbech, N.P. Analysis of microbial communities with electrochemical microsensors and microscale biosensors. *Methods Enzymol.* **2005**, *397*, 147. [PubMed]
21. Revsbech, N.P.; Risgaard-Petersen, N.; Schramm, A.; Nielsen, L.P. Nitrogen transformations in stratified aquatic microbial ecosystems. *Antonie Van Leeuwenhoek* **2006**, *90*, 361–375. [CrossRef] [PubMed]
22. Hesslein, R.H. An in situ sampler for close interval pore water studies. *Limnol. Oceanogr.* **1976**, *21*, 912–914. [CrossRef]

23. Mayer, L.M. Chemical Water Sampling in Lakes and Sediments with Dialysis Bags. *Limnol. Oceanogr.* **1976**, *21*, 909–912. [CrossRef]
24. Mosier, A.C.; Francis, C.A. Relative abundance and diversity of ammonia-oxidizing archaea and bacteria in the San Francisco Bay estuary. *Environ. Microbiol.* **2008**, *10*, 3002. [CrossRef] [PubMed]
25. Chen, M.; Ding, S.; Liu, L.; Xu, D.; Han, C.; Zhang, C. Iron-coupled inactivation of phosphorus in sediments by macrozoobenthos (chironomid larvae) bioturbation: Evidences from high-resolution dynamic measurements. *Environ. Pollut.* **2015**, *204*, 241–247. [CrossRef] [PubMed]
26. Aller, R.C.; Aller, J.Y. The effect of biogenic irrigation intensity and solute exchange on diagenetic reaction rates in marine sediments. *J. Mar. Res.* **1998**, *56*, 905–936. [CrossRef]
27. Walshe, B.M. Feeding Mechanism of *Chironomus* larvae. *Nature* **1947**, *160*, 474. [CrossRef] [PubMed]
28. Lewandowski, J.; Laskov, C.; Hupfer, M. The relationship between *Chironomus plumosus* burrows and the spatial distribution of pore-water phosphate, iron and ammonium in lake sediments. *Freshw. Biol.* **2007**, *52*, 331–343. [CrossRef]
29. Caffrey, J.M.; Kemp, W.M. Influence of the submersed plant, *Potamogeton perfoliatus*, on nitrogen cycling in estuarine sediments. *Limnol. Oceanogr.* **1992**, *37*, 1483–1495. [CrossRef]
30. Roskosch, A.; Hette, N.; Hupfer, M.; Lewandowski, J. Alteration of *Chironomus plumosus* ventilation activity and bioirrigation-mediated benthic fluxes by changes in temperature, oxygen concentration, and seasonal variations. *Freshw. Sci.* **2012**, *31*, 269–281. [CrossRef]
31. Charpentier, J.; Martin, G.; Wacheux, H.; Gilles, P. ORP regulation and Activated Sludge: 15 years of experience. *Water Sci. Technol.* **1998**, *38*, 197–208.
32. Li, B.; Bishop, P. Oxidation-reduction potential (ORP) regulation of nutrient removal in activated sludge wastewater treatment plants. *Water Sci. Technol.* **2002**, *46*, 35–38. [PubMed]
33. Bertics, V.; Sohm, J.; Treude, T.; Chow, C.E.T.; Capone, D.G.; Fuhrman, J.A.; Ziebis, W. Burrowing deeper into benthic nitrogen cycling: The impact of bioturbation on nitrogen fixation coupled to sulfate reduction. *Mar. Ecol. Progress* **2010**, *409*, 1–15. [CrossRef]
34. Pelegrí, S.P.; Blackburn, T.H. Nitrogen cycling in lake sediments bioturbated by *Chironomus plumosus* larvae, under different degrees of oxygenation. *Hydrobiologia* **1996**, *325*, 231–238. [CrossRef]
35. Svensson, J.M. Influence of *Chironomus plumosus* larvae on ammonium flux and denitrification (measured by the acetylene blockage- and the isotope pairing-technique) in eutrophic lake sediment. *Hydrobiologia* **1997**, *346*, 157–168. [CrossRef]