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N2O Fluxes and Rates of Nitrification and Denitrification at the Sediment–Water Interface in Taihu Lake, China

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Abstract: Because of global concerns regarding pollution and eutrophication in fresh water, China’s Taihu Lake has gained attention both for these issues and as a source of nitrous oxide (N2O) emissions. In this study, we investigated N2O fluxes and nitrification and denitrification rates at the sediment–water interface and analyzed monthly the relationships between these processes in different areas of Taihu Lake over a one-year period. Annual maximum nitrification and denitrification rate and N2O flux were observed during June in an algae-dominated area of the lake and measured 17.80, 235.51, and 31.49 µmol N m−2 h−1, respectively. The nitrification rate ranged from 0 to 1.18 µmol N m−2 h−1 at other sampling sites, with less variation. The denitrification rate showed clear seasonal variation, with lower levels between August and January (0.01–8.57 µmol N m−2 h−1; average = 1.49 µmol N m−2 h−1) and a rapid increase between February and July (1.03–235.51 µmol N m−2 h−1; average = 41.73 µmol N m−2 h−1). The N2O flux ranged from −0.64 to 1.5 µmol N m−2 h−1, with little variability except for a much higher rate (31.49 µmol N m−2 h−1) in June in algae-dominated areas. N2O flux was significantly positively correlated with nitrification and denitrification rates in most lake zones. By comparing the slopes of the regression equations, we found that N2O emissions from the sediment–water interface were influenced predominantly by nitrification, suggesting that lower N2O fluxes from the sediment–water interface in Taihu Lake are caused primarily by lower nitrification rates.

Keywords: nitrous oxide flux; nitrification; denitrification; sediment–water interface; Taihu Lake China

1. Introduction

Nitrous oxide (N2O) is the third important greenhouse gas, and has a greenhouse effect approximately 300 times stronger than that of CO2 [1]. The atmospheric N2O concentration has kept increasing steadily over the past century by approximately 0.25 ± 0.05% year−1 (IPCC, 2007), which has gained great attention. Water eutrophication caused by excess phosphorus has become a severe environmental problem globally, while excessive nitrogen accumulation in water produces nitrous oxide (N2O) emissions, exacerbating the greenhouse effect [2,3]. Although lakes are not generally considered major sources of N2O emissions compared to other aquatic ecosystems such
as rivers and wetlands [4], increasing levels of pollution and eutrophication in freshwater lakes can cause ongoing problems, including higher N$_2$O concentrations, saturation, and emissions fluxes. Nitrification and denitrification are among the microbial processes that produce N$_2$O in lakes and other aquatic ecosystems [5–7]. A byproduct of microbial nitrification and denitrification processes, N$_2$O can also be consumed by denitrification [8]; net N$_2$O emissions may therefore result from these two dynamic processes in which N$_2$O is both produced and consumed in lake ecosystems [9]. Nevertheless, opinions differ regarding the dominant N$_2$O emission process at the sediment–water interface in various ecological environments [10]. The sediment–water interface, which plays an important role in the removal and transformation matter, is an important part of rivers, lakes, and other aquatic ecosystems [11]. Material cycling processes occurring at the sediment–water interface in lakes have recently attracted increasing attention from researchers. Most studies on the exchange and migration of nutrients [12–14] and heavy metals [15,16] at the sediment–water interface have been performed in aquatic ecosystems. Several studies have specifically investigated nitrification, denitrification, and N$_2$O emissions at this interface [17,18]. Xia et al. [19] found that the sediment–water interface denitrification rate increased with ammonia concentration in the water. Jha and Masao [20] determined that water column nitrate concentration and temperature were the primary factors influencing the denitrification rate in Barato Lake, Japan. Teixeira et al. [21] found that N$_2$O emissions at the sediment–water interface increased with seawater salinity. Kenny et al. [22] found a strong positive correlation between N$_2$O emission rates and ammonium concentration in a study concerning sediment nitrate concentrations in water, while Beaulieu et al. [23] found no relationship between N$_2$O yield and aquatic N-NO$_3$ in American streams.

Significantly different nitrification and denitrification rates and N$_2$O emissions have been reported at the sediment–water interface in different aquatic ecosystems due to high ecosystem complexity and differences between given locations: such differences can occur even between two bodies of water in the same location [24,25]. In this study, from August 2014 to July 2015, we examined nitrification and denitrification rates and N$_2$O fluxes at the sediment–water interface in Taihu Lake, a large and important lake in China where few studies [20–22] have focused on these processes, allowing us to determine the dominant N$_2$O emission process.

2. Materials and Methods

2.1. Study Site

Taihu Lake is the third largest freshwater lake in China (2338 km$^2$) [26], located in eastern China. Taihu is a shallow lake with a mean depth of 2 m and a bed ranging between 1.5 m and 2.5 m (72% of total area). Taihu Lake serves economically developed regions with high-density populations and well-developed industries near the Changjiang (Yangtze) River delta (Figure 1). These developed regions have contributed excessive inputs of nitrogen and phosphorus [27,28]. In 2012, the nutrients load exceeded 0.93 g P m$^{-2}$ year$^{-1}$ and 19 g N m$^{-2}$ year$^{-1}$ [29], which contribute in turn to ongoing eutrophication. The lake is characterized by different ecological zones, such as algal zones, macrophyte zones, and open-water zones with high spatial variation [30].

We selected four sampling locations within the lake (Figure 1). Average total P concentration in the lake is 0.14 mg P L$^{-1}$ with the range of 0.02 mg P L$^{-1}$ (July) to 0.57 mg P L$^{-1}$ (April), which indicates poor water quality [31] and lies in water quality standard IV grade described by China environmental bulletin 2014. The first sampling site (S1), located in Meiliang Bay, is considered a typical algal zone. This site has an average water depth of about 3.2 m, with the range of 3 m (January and February) to 3.7 m (July, August and September) [32]. It features significant amounts of sludge on the lake bottom; dense algal blooms occur from June to August; and the level of eutrophication is highest at S1, as noted by Yang et al. [33] and Wang et al. [34]. The second sampling site (S2) is situated in an open-water zone with an average water depth of about 2 m, where the sediment contains more sand and no aquatic plants, with much algae in water. The other two sites are located in the eastern portion of the lake.
with an average water depth of about 1.6 m, which is dominated by large aquatic plants. The third sampling site (S3) is characterized by floating plants and the fourth sampling site (S4) by submerged plants, which produce better water quality [35]. Thus, the sediments contain more organic detritus. The chemical characteristics of the water and sediment at all four sites are given in Tables 1 and 2.

![Sampling sites S1, S2, S3, and S4 in Taihu Lake.](image)

**Figure 1.** Sampling sites S1, S2, S3, and S4 in Taihu Lake.

**Table 1.** Chemical characteristics of water samples (mg L\(^{-1}\)). Analytical results over one year for ammonia (N-NH\(_4\)), nitrate (N-NO\(_3\)), total nitrogen (TN), total dissolved nitrogen (TDN), chemical oxygen demand (COD), and chlorophyll a (Chl a) in water samples from the four sampling locations. Data are given as concentration ranges with the annual average in parentheses. For all parameters except for Chl a, the annual maximum occurred in June at S1.

| Site | N-NH\(_4\) | N-NO\(_3\) | TN | TDN | COD\(_{Mn}\) | Chl a |
|------|------------|------------|----|-----|-------------|-------|
| S1   | 0.07–0.57  | 0.33–2.38  | 0.67–4.51 | 0.61–3.18 | 4.8–11.0 | 5.3–70.7 |
|      | (0.23)     | (0.74)     | (1.85) | (1.16) | (7.3)       | (27.1) |
| S2   | 0.09–0.29  | 0.27–1.55  | 0.79–3.23 | 0.46–2.09 | 4.3–7.8 | 2.3–89.6 |
|      | (0.20)     | (0.90)     | (1.78) | (1.31) | (6.0)       | (17.0) |
| S3   | 0.09–0.37  | 0.22–1.11  | 0.52–1.71 | 0.45–1.37 | 3.7–7.1 | 0.14–14.1 |
|      | (0.19)     | (0.56)     | (1.06) | (0.89) | (4.8)       | (5.3)  |
| S4   | 0.06–0.37  | 0.21–1.16  | 0.42–1.88 | 0.34–1.51 | 3.7–7.1 | 1.1–11.1 |
|      | (0.19)     | (0.55)     | (1.01) | (0.83) | (4.9)       | (4.3)  |

**Table 2.** Chemical characteristics of sediment (mg kg\(^{-1}\) dry weight). Analytical results over one year for ammonia (N-NH\(_4\)), nitrate (N-NO\(_3\)), nitrite (N-NO\(_2\)), and total nitrogen (TN) concentrations, along with organic carbon (Org C) content, in sediment samples from the four sampling locations. Data are given as concentration ranges with the annual average in parentheses.

| Site | N-NH\(_4\) | N-NO\(_3\) | N-NO\(_2\) | TN     | Org C (%) |
|------|------------|------------|------------|--------|-----------|
| S1   | 0.6–226.1  | 11.3–47.8  | 0.14–0.48  | 1747–2832 | 1.06–1.53 |
|      | (57.1)     | (25.0)     | (0.23)     | (2259) | (1.27)    |
| S2   | 10.3–134.7 | 9.1–51.6   | 0.11–0.54  | 1344–2188 | 0.85–1.34 |
|      | (52.5)     | (23.8)     | (0.23)     | (1674) | (1.05)    |
| S3   | 8.7–87.2   | 7.5–52.2   | 0.09–0.47  | 1666–2431 | 1.26–2.34 |
|      | (35.8)     | (25.9)     | (0.24)     | (1893) | (1.66)    |
| S4   | 2.1–207.4  | 16.9–51.1  | 0.16–0.45  | 1397–2292 | 1.27–2.23 |
|      | (38.7)     | (25.9)     | (0.26)     | (1930) | (1.63)    |
2.2. Sampling Procedure

We collected samples monthly from August 2014 to July 2015. The sites were located using a GPS device. Fifteen sediment–water column samples were collected at each site using a gravity sampler 9 cm in diameter and 30 cm in height, which produced a 20 cm sediment core with 10 cm of overlying water. Water temperature was determined simultaneously using multi-parameter water probe meters (YSI Inc., Yellow Springs, OH, USA). Twelve samples were used to determine the nitrification and denitrification rates and N\textsubscript{2}O flux at the sediment–water interface; the uppermost 2 cm of sediment in the remaining three samples was collected and sealed in polyethylene plastic bags, which were subsequently flattened to keep the sediment in an anaerobic state for analysis. In addition, 10 L samples of overlying water were collected; 50 mL aliquots of which were filtered through a 0.45 µm Millipore membrane filter for the determination of ammonia and nitrate. The 50 mL water and sediment samples were placed in an incubator with ice packs, transported to the Chinese Academy of Sciences Taihu Lake Ecological Experiment Station as soon as possible, and stored in a refrigerator until tests could be completed. The sediment–water columns were placed in an Electro-Thermostatic sink (STICK Inc., Shanghai, China) to regulate the water temperature, which would maintain the value measured in the lake and maximally simulate the actual state of lake. It is a little different with in situ incubations in benthic chambers [36]. Intact sediment cores were incubated in a laboratory microcosm usually used to study the effect of environmental factors on greenhouse fluxes across sediment water interface [18,19]. Thus, it could meet to the requirement of this research.

2.3. Analytical Procedures

The nitrification and denitrification rates and N\textsubscript{2}O flux were determined as follows. The sediment–water column samples were divided into four groups. In the first group, 60 mL serum bottles were filled with overlying water using a siphon before the incubation experiment began. Then, 0.5 mL of saturated HgCl\textsubscript{2} was added and the bottles were sealed. In the second group, used as a control group, no inhibitors were added. The third group was treated with acetylene to prevent the N\textsubscript{2}O from being converted into N\textsubscript{2}. Saturated acetylene solution was added to the sediment–water column in amounts sufficient to achieve 10% acetylene concentrations in both the porewater and the overlying water [37]. This group was used to determine the denitrification rate. The fourth group was treated with the nitrification inhibitor allylthiourea (ATU), which prevents the microbial oxidation of N-NH\textsubscript{4} to N-NO\textsubscript{3} and N\textsubscript{2}O produced from nitrification [38,39]. ATU was added to the sediment–water column in amounts sufficient to achieve a concentration of 10 mg L\textsuperscript{-1} in both the porewater and the overlying water to determine the nitrification rate. A multi-channel peristaltic pump (BT100-L Baoding Lange) was used to keep the water flowing and ensure evenly distributed nutrient content. An incubation time of 4 h in the dark was used throughout the process, after which 60 mL serum bottles were filled with overlying water from sediment water column using a siphon. The 0.5 mL aliquots of saturated HgCl\textsubscript{2} were added to the serum bottles to inhibit microbial activity. Finally, the solution in the serum bottles was used to determine the amount of dissolved N\textsubscript{2}O.

2.4. Environmental Variables and N\textsubscript{2}O Measurement

Chemical characteristics of water and sediment were determined using standard methods [40]. We determined water and sediment ammonia nitrogen (N-NH\textsubscript{4}) using Nessler’s reagent colorimetric method (LOD of 0.02 mg N L\textsuperscript{-1}) and nitrate nitrogen (N-NO\textsubscript{3}) using UV spectrophotometry (LOD of 0.02 mg N L\textsuperscript{-1}). Total dissolved nitrogen (TDN) and total nitrogen (TN) were determined using the alkaline potassium persulfate digestion-UV spectrophotometry method (LOD of 0.05 mg N L\textsuperscript{-1}). Nitrite nitrogen (N-NO\textsubscript{2}) was determined using the Griess spectrophotometry method (LOD of 0.003 mg N L\textsuperscript{-1}). Chemical oxygen demand (COD\textsubscript{Mn}) was determined using the potassium permanganate method. Chlorophyll a (Chl a) was determined using
the ethanol extraction-spectrophotometry method. The amount of organic carbon (Org-C) in sediment samples was determined using the potassium dichromate oxidation-ferrous sulfate titrimentry method.

We determined the concentration of dissolved $N_2O$ using the headspace equilibrium technique [32], in which 10 mL of high purity $N_2$ was injected into a serum bottle to displace 10 mL of water. The bottle was then shaken vigorously for 30 min at room temperature until the liquid and gas phases reached equilibrium. Then, the headspace gas was extracted with an ejector and the concentration of $N_2O$ was determined via gas chromatography (GC) with electron capture detection (ECD) (Agilent 7890B), during which the column temperature was 60 °C, the ECD detector temperature was 300 °C, and 99.999% grade $N_2$ carrier gas was used at a flow rate of 35 mL min$^{-1}$. The water $N_2O$ concentration was calculated using formulas derived by Johnson et al. [41], and data concerning the solubility of $N_2O$ in water was drawn from Weiss and Price [42]. Treatment with ATU allows the indirect calculation of the contribution of nitrification to the $N_2O$ flux, which is expressed as the difference between the control group and the ATU treatment group [38,39,43]. The denitrification rate was calculated using the $N_2O$ accumulation in the acetylene treatment group. The $N_2O$ flux at the sediment–water interface was then calculated using the difference in $N_2O$ concentration between the control group and the initial sample. This method is similar to a laboratory-scale benthic chamber [44], which is also an accurate and direct way for determining the $N_2O$ flux across sediment–water interface in comparison with the gradient method [45].

2.5. Statistical Analysis

All data analysis was performed using SPSS 19.0 and Microsoft Excel 2007 software in Windows 7. Simple linear regressions were used to describe the relationships between the $N_2O$ flux at the sediment–water interface and the nitrification and denitrification rates. The relationship was considered statically significant at values of $p < 0.05$.

3. Results

3.1. Chemical Characteristics of Water and Sediment

The ranges and average values for the relevant water and sediment chemical characteristics over the one-year study period are presented in Tables 1 and 2. The water-borne concentrations of TN, $N-NH_4$, COD$_{MN}$, and Chl a were generally higher and more variable at S1 than at the other sites (Table 1). $N-NO_3$ was the dominant form of inorganic nitrogen. The concentrations of $N-NH_4$ in the water varied far less than did the other variables. Overall, the algal productivity in S1 and S2 zones were higher than those in the macrophyte zones (S3 and S4). The sediment-borne $N-NO_3$ concentrations were lower than those of ammonia ($N-NH_4$) on average (Table 2), and the amounts of sedimentary $N-NO_2$ were small. The concentrations of TN at S1 were higher than those at the other sites. The macrophyte zones (S3 and S4) had higher Org-C contents than S1 and S2.

3.2. Nitrification and Denitrification Rates at the Sediment–Water Interface

The monthly nitrification and denitrification rates at the sediment–water interface are shown in Figure 2. The annual maximum values at S1 were recorded in June, and both maxima were significantly higher than the other values. At S2, the nitrification rate was maximum in January and April, however the other sites featured no major annual variations. Overall, the nitrification rates were low, with little seasonal variation.

There were no significant differences in denitrification rate between the sampling sites from August 2014 to January 2015. However, denitrification rates increased with increasing water temperature (February 2015 to July 2015) and denitrification rates increased most noticeably at S1. Most of the sampling sites maintained elevated denitrification rates through July. Overall, the denitrification rates were higher than the nitrification rates.
3.3. Monthly Variations in N$_2$O Flux at the Sediment–Water Interface

Monthly variations in the N$_2$O flux at the sediment–water interface are shown in Figure 3. At S1, the minimum and maximum N$_2$O fluxes were observed in November and June, respectively. At S2, the N$_2$O fluxes, which surpassed those at any other site, were highest in April and May. Overall, the N$_2$O fluxes varied over a narrow range for all sites except S1 and remained low during most of the year.

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**Figure 2.** Monthly variations in nitrification and denitrification rates and water temperature at the sediment–water interface in Taihu Lake.

**Figure 3.** Monthly variations in the N$_2$O flux at the sediment–water interface.
4. Discussion

4.1. The Influence of Environmental Variables on Nitrification at the Sediment–Water Interface

The nitrogen content in lake water has a direct effect on the nitrification process. The TN concentration measured in this study was lower than that in previous years [46], which resulted from recent environmental management, such as sediment dredging [47]. The lower TN concentration (Table 1) could be the primary cause of the lower nitrification rates observed throughout the study period (Figure 2a). The correlation analysis presented in Table 3 shows significant positive correlations between nitrification rate and water N-NH$_4$, N-NO$_3$, TN, and TDN ($p < 0.01$). These results indicate that dissolved nitrogen significantly affected the nitrification rate at the sediment–water interface, which is consistent with previous findings [48]. Since the content of Chl a is closely related to the algal productivity, Chl a was positively correlated with both the nitrification rate and the N$_2$O flux ($p < 0.01$), which confirms that algal blooms may promote nitrification and N$_2$O production [49,50].

Although temperature is important and can affect nitrifying microorganism activity, thus affecting the nitrification rate [51–53], no clear relationship was found between water temperature and nitrification rate in this study (Table 3) (Figure 2a). Overall, the nitrification rate at the sediment–water interface fluctuated over a narrow range at all Taihu Lake sampling sites, except S1. Thus, the nitrification rate was low throughout most of the year and lower than that reported in estuary sediments by Yang et al. [43]. Therefore, nitrification at the sediment–water interface in Taihu Lake may have been limited by the lower water nitrogen concentrations.

4.2. The Influence of Environmental Variables on Denitrification at the Sediment–Water Interface

In a lacustrine ecosystem such as Taihu Lake, denitrification occurs mainly in sediment and is affected by factors such as nitrate concentration [6], temperature [54], and organic carbon content [55,56]. In this study, the denitrification rate was positively correlated with the water N-NO$_3$, TN, and TDN concentrations ($p < 0.01$) (Table 3), but there were no obvious relationships between the denitrification rate and the various sediment characteristics (Table 4). This indicates that the denitrification rate at the sediment–water interface increases with increased dissolved N loading in Taihu Lake. The rivers, especially during the high rainfalls mainly concentrated in June, flows nutrients from cultivated fields, urban soil surfaces, and domestic waste exudates into Meiliang Bay (S1) [57–59], which rapidly increase both the nitrogen content in the lake water reaching the yearly maximum and the rate of denitrification (Figure 2b).

Table 3. Correlations among N$_2$O flux, nitrification rate, denitrification rate, and water parameters.

| Parameters | N-NH$_4$ | N-NO$_3$ | TN | TDN | COD$_{Mn}$ | Chl a | Nitr. Rate | Denitr. Rate | N$_2$O Flux | T |
|------------|----------|----------|----|-----|------------|-------|------------|-------------|-------------|----|
| N-NH$_4$   | 1        | 0.160    | 0.158 | 0.103 | 0.224 | 0.183 | 0.523 ** | 0.242 | 0.496 ** | −0.07 |
| N-NO$_3$   | 1        | 0.841 ** | 0.935 ** | 0.017 | 0.314 * | 0.611 ** | 0.617 ** | 0.060 |
| TN         | 1        | 0.884 ** | 0.235 | 0.480 ** | 0.613 ** | 0.676 ** | 0.618 ** | 0.79 |
| TDN        | 1        | −0.044   | 0.276 | 0.581 ** | 0.718 ** | 0.603 ** | 0.032 |
| COD$_{Mn}$ | 1        | 0.662 ** | 0.148 | 0.019 | 0.118 | 0.211 |
| Chl a      | 1        | 0.390 ** | 0.216 | 0.391 ** | 0.178 |
| Nitr. rate | 1        | 0.766 ** | 0.996 ** | −0.074 |
| Denitr. rate | 1       | 0.782 ** | 0.042 |
| N$_2$O Flux | 1       | −0.072 |
| T          | 1        |

Notes: ** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed). These designations also hold true for Table 4.
Table 4. Correlations among N₂O flux, nitrification rate, denitrification rate, and sediment parameters.

| Parameters | N-NH₄ | N-NO₃ | N-NO₂ | TN  | Org-C | Nitr. Rate | Denitr. Rate | N₂O Flux |
|------------|-------|-------|-------|-----|-------|------------|-------------|----------|
| N-NH₄      | 1     |       |       | 0.19|       | −0.066     | 0.076       | 0.176    |
| N-NO₃      | 1     |       |       | −0.150|      | 0.115     | 0.0366 *    | 0.097    |
| N-NO₂      | 1     |       |       | 0.160|      | −0.085    | −0.066    | 0.766 ** |
| TN         | 1     |       |       | 0.366 *|      | −0.097    | −0.085     | 0.966 ** |
| Org-C      | 1     |       |       | 0.366 *|      | −0.097    | −0.085     | 0.966 ** |
| Nitr. rate |       |       |       |      | 1     | −0.085    | −0.086     | 0.782 ** |
| Denitr. rate|     |       |       |      | 1     |          | -          |          |
| N₂O flux   |       |       |       |      |       |           |            |          |

Notes: ** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).
These designations also hold true for Table 4.

Many studies have found that the denitrification rate rises with increasing water temperature [43–45]. In this study, this pattern appeared to occur between February and June 2015, and no obvious changes occurred from August 2014 to January 2015 (Figure 2b). Our correlation analysis showed that water temperature had no significant effect on the denitrification rate (Table 3), suggesting that the effects of water temperature on the denitrification rate vary between regions and seasons [54,60]. The lower denitrification rates observed in this study in autumn and winter suggest that denitrification may be inhibited by the lower levels of nitrate in sediment and water, sediment organic carbon content, and decreased microbial activity [61]. On the other hand, the sedimentation of dead algae and hydrophyte residues increased in spring and summer, which increased the rate of nitrogen utilization by microorganisms [62]. These factors combined to rapidly increase the denitrification rate during spring and summer. Overall, these results suggest that denitrification is influenced primarily by seasonal changes. However, nitrification rate showed no seasonal variations, which is perhaps limited by the lower water N-NH₄ concentrations (Table 1).

4.3. N₂O Production Mechanisms at the Sediment–Water Interface in Taihu Lake

N₂O can be produced from several different processes, including nitrification, denitrification, dissimilatory nitrate reduction to ammonium (DNRA) [63], and some chemical processes [64]. However, in aquatic ecosystems, researchers remain concerned about the effects of nitrification and denitrification on N₂O production. Our correlation analysis found close relationships between the N₂O flux and the rates of nitrification and denitrification (Table 3) (p < 0.01), which indicates that these processes had a significant effect on N₂O flux at the sediment–water interface in Taihu Lake.

In lakes, nitrification can occur in both the water column and the surface sediment [63]. Furthermore, denitrification usually occurs in an anaerobic sediment layer [65–67]. N₂O fluxes are thought to arise primarily from denitrification [68], but the N₂O flux is also closely related to nitrification in aquatic ecosystems [67,69]. In this study, we investigated the response of the N₂O flux to the nitrification and denitrification rates using regression analysis (Figure 4). Quite significant positive correlations with obviously different slopes were found between the N₂O flux and the nitrification rate at S1, S2, and S4 (p < 0.01). Thus, the results indicate that the N₂O production by nitrification was more rapid at S1 than at other sites. The correlation between the N₂O flux and the denitrification rate was highly significant at S1, S3, and S4. However, the slopes suggest that denitrification did not have a significant effect on the N₂O flux at the sediment–water interface in Taihu Lake, which indicate that nitrification may play a key role in N₂O flux. Moreover, pervious references evidenced that less N₂O produced by denitrification and more N₂O by nitrification were released to the overlying water [25,48,67]. These results are consistent with the present research. In view of inhibitor methods’ limitation [70,71], it is difficult to determine the amount of N₂O production via nitrification and denitrification. Thus, further research is needed.
Figure 4. Relationships between nitrification and denitrification rates and N\textsubscript{2}O flux at the sediment–water interface, including the regression equation, the sample number (n), and the coefficient of determination (R\textsuperscript{2}), accompanied by p values, at each site.

As most allochthonous nutrient comes from the north of the Taihu Basin with most cities and the major inflow rivers [72], high nutrient concentrations in water can be observed at Meiliang Bay.
As most allochthonous nutrient comes from the north of the Taihu Basin with most cities and the major inflow rivers [72], high nutrient concentrations in water can be observed at Meiliang Bay (S1) in the beginning of rainy season (June), further releasing more N$_2$O. However, water nutrient concentration would be diluted due to much rainfall during monsoon season (August and September) [32] and due to continuous nutrient consumption by enlarging algae [73], reducing nitrification and denitrification rates and N$_2$O flux (Figures 2 and 3). This is likely the main reason that the nitrification and denitrification rates and N$_2$O flux peaked in June 2015 at S1 (Figures 2 and 3). Other than the nutrient increases at S1 in June 2015, no significant differences in nutrients were found at any of the sampling sites in any month. These results suggest that significant changes in water nutrient concentration can significantly affect the nitrification and denitrification rates and N$_2$O flux at the sediment–water interface. However, smaller water nutrient concentration fluctuations were clearly not sufficient to produce significant changes in Taihu Lake.

5. Conclusions

This study investigated seasonal changes in the nitrification and denitrification rates and N$_2$O flux at the sediment–water interface in Taihu Lake, China. Our results show that the nitrification rate was both lower and less variable than the denitrification rate and did not feature seasonal variation (other than one outlier at one sampling site), suggesting that nitrification was limited by the lower concentrations of dissolved nitrogen. The denitrification rate featured more obvious seasonal variation and a much higher annual average, but did not cause increased N$_2$O emissions at the sediment–water interface. Our analysis suggests instead that N$_2$O emissions from the sediment–water interface may be dominated by nitrification; thus, the lower N$_2$O flux from the sediment–water interface in Taihu Lake was due primarily to lower nitrification rates. Nitrification and denitrification are affected by many factors, and the mechanisms of N$_2$O production are equally complex. Future experiments should focus on identifying these factors via experiments under various conditions and more advanced technology should be adopted to determine the amount of N$_2$O production via nitrification and denitrification.

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References

1. Ravishankara, A.R.; Daniel, J.S.; Portmann, R.W. Nitrous oxide (N$_2$O): The dominant ozone-depleting substance emitted in the 21st century. Science 2009, 326, 123–125. [CrossRef] [PubMed]
2. Corredor, J.E.; Morell, J.M.; Bauza, J. Atmospheric nitrous oxide fluxes from mangrove sediments. Mar. Pollut. Bull. 1999, 38, 473–478. [CrossRef]
3. Huttunen, J.T.; Alm, J.; Liikanen, A.; Juutinen, S.; Larmola, T.; Hammar, T.; Silvola, J.; Martikainen, P.J. Fluxes of methane, carbon dioxide and nitrous oxide in boreal lakes and potential anthropogenic effects on the aquatic greenhouse gas emissions. Chemosphere 2003, 52, 609–621. [CrossRef]
4. Seitzinger, S.P.; Kroeze, C. Global distribution of nitrous oxide production and N inputs in freshwater and coastal marine ecosystems. Glob. Biogeochem. Cycle 1998, 12, 93–113. [CrossRef]
5. Hou, L.J.; Liu, M.; Xu, S.Y.; Ou, D.N.; Yu, J.; Cheng, S.B.; Lin, X.; Yang, Y. The effects of semi-lunar spring and neap tidal change on nitrification, denitrification and N$_2$O vertical distribution in the intertidal sediments of the Yangtze estuary, China. Coast. Shelf Sci. 2007, 73, 607–616. [CrossRef]
6. Seitzinger, S.P. Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnol. Oceanogr.* **1988**, *33*, 702–724. [CrossRef]

7. Zhao, Y.; Xia, Y.; Li, B.; Yan, X. Influence of environmental factors on net N₂ and N₂O production in sediment of freshwater rivers. *Environ. Sci. Pollut. Res.* **2014**, *21*, 9973–9982. [CrossRef] [PubMed]

8. Xu, Y.; Cai, Z.; Xu, Z. Production and consumption of N₂O during denitrification in subtropical soils of China. *J. Soil. Sediment* **2012**, *12*, 1339–1349. [CrossRef]

9. Bange, H.W.; Spyridon, R.; Andreae, M.O. Nitrous oxide in coastal waters. *Glob. Biogeochem. Cycle* **1996**, *10*, 197–207. [CrossRef]

10. Gao, Y.; Zhang, Z.H.; Liu, X.H.; Yi, N.; Zhang, L.; Song, W.; Wang, Y.; Mazumder, A.; Yan, S.H. Seasonal and diurnal dynamics of physio chemical parameters and gas production in vertical water column of a eutrophic pond. *Ecol. Eng.* **2016**, *87*, 313–323. [CrossRef]

11. Santschi, P.; Höhener, P.; Benoit, G.; Brink, B.T. Chemical processes at the sediment-water interface. *Mar. Chem.* **1990**, *30*, 269–315. [CrossRef]

12. Yang, L.; Cai, Q.; Qin, B.; Zheng, X. Characteristics of nitrogen removing between sediment-water interface in Meiliang Bay, Taihu Lake. *J. Lakeecence* **1998**, *10*, 41–47.

13. Boström, B.; Andersen, J.M.; Fleischer, S.; Jansson, M. Exchange of phosphorus across the sediment-water interface. *Hydrobiologia* **1988**, *170*, 229–244. [CrossRef]

14. Helali, M.A.; Zaaboub, N.; Oueslati, W.; Added, A.; Aleya, L. Nutrient exchange and oxygen demand at the sediment-water interface during dry and wet seasons of the Medjerda river delta (Tunis Gulf, Tunisia). *Environ. Earth Sci.* **2016**, *75*, 25. [CrossRef]

15. Petersen, W.; Wallman, K.; Pinglin, L.; Schroeder, F.; Knauth, H.D.; Petersen, W.; Wallman, K.; Pinglin, L.; Schroeder, F.; Knauth, H.D. Exchange of trace elements at the sediment-water interface during early diagenesis processes. *Mar. Freshw. Res.* **1995**, *46*, 19–26.

16. Hellali, M.A.; Zaaboub, N.; Oueslati, W.; Added, A.; Aleya, L. Diagenetic processes and sediment-water exchanges of heavy metals in the Mejerda river delta (Gulf of Tunis). *Environ. Earth Sci.* **2015**, *74*, 6665–6679. [CrossRef]

17. Katharina, F.; Martin, A.; Hans-Peter, G.; Werner, E. Microbial response to experimentally controlled redox transitions at the sediment water interface. *PLoS ONE* **2015**, *10*, e0143428.

18. Liikanen, A.; Martikainen, P.J. Effect of ammonium and oxygen on methane and nitrous oxide fluxes across sediment–water interface in a eutrophic lake. *Chemosphere* **2003**, *52*, 1287–1293. [CrossRef]

19. Xia, X.; Li, S.; Shen, Z. Effect of nitrification on nitrogen flux across sediment–water interface. *Water Environ. Res.* **2008**, *80*, 2175–2182. [CrossRef] [PubMed]

20. Jha, P.K.; Masao, M. Factors affecting denitrification rate in Barato Lake, Hokkaido, Japan. *Asian J. Water Environ. Pollut.* **2011**, *3*, 1–10.

21. Teixeira, C.; Magalhaes, C.; Joye, S.B.; Bordalo, A.A. The role of salinity in shaping dissolved inorganic nitrogen and N₂O dynamics in estuarine sediment-water interface. *Mar. Pollut. Bull.* **2013**, *66*, 225–229. [CrossRef] [PubMed]

22. Kenny, C.; Yamulki, S.; Blackwell, M.; Maltby, E.; French, P.; Birgand, F. The release of nitrous oxide from the intertidal zones of two European estuaries in response to increased ammonium and nitrate loading. *Water Air Soil Pollut.* **2005**, *164*, 61–66. [CrossRef]

23. Beaulieu, J.J.; Tank, J.L.; Hamilton, S.K.; Wollheim, W.M.; Hall, R.O.; Mulholland, P.J.; Peterson, B.J.; Ashkenas, L.R.; Cooper, L.W.; Dahm, C.N. Nitrous oxide emission from denitrification in stream and river networks. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 214–219. [CrossRef] [PubMed]

24. Wang, S.L.; Yeager, K.M.; Wan, G.J.; Liu, C.Q.; Tao, F.X.; Fan, C.X. Short-term field observations of nitrous oxide saturations in Lake Taihu, China: The need for high temporal resolution studies. *J. Environ. Qual.* **2010**, *39*, 1858–1863. [CrossRef] [PubMed]

25. Wang, H.; Yang, L.; Wang, W.; Lu, J.; Yin, C. Nitrous oxide (N₂O) fluxes and their relationships with water-sediment characteristics in a hyper-eutrophic shallow lake, China. *J. Geophys. Res.* **2007**, *112*, 129–137. [CrossRef]

26. Qin, B.Q.; Xu, P.Z.; Wu, Q.L.; Luo, L.C.; Zhang, Y.L. Environmental issues of lake Taihu, China. *Hydrobiologia* **2007**, *581*, 3–14. [CrossRef]

27. Yang, L.Y.; Qin, B.Q.; Hu, W.P.; Luo, L.C.; Song, Y.Z. The atmospheric deposition of nitrogen and phosphorus nutrients in TaihuLake. *Oceanol. Et Limnol. Sin.* **2007**, *38*, 104–110.
28. Chen, X.; Fei, W.; Chen, C.; Fang, K. Seasonal changes in the concentrations of nitrogen and phosphorus in farmland drainage and groundwater of the Taihu Lake region of China. *Environ. Monit. Assess.* 2010, 169, 159–168. [CrossRef] [PubMed]

29. Xu, H.; Paerl, H.W.; Qin, B.Q.; Zhu, G.W.; Hall, N.S.; Wu, Y.L. Determining critical nutrient thresholds needed to control harmful cyanobacterial blooms in eutrophic Lake Taihu, China. *Environ. Sci. Technol.* 2015, 49, 1051–1059. [CrossRef] [PubMed]

30. Janssen, A.B.G.; Teurlincx, S.; An, S.Q.; Janse, J.H.; Paerl, H.W.; Mooij, W.M. Alternative stable states in large shallow lakes? *J. Gt. Lakes Res.* 2014, 40, 813–826. [CrossRef]

31. Dong, X.H.; Bennion, H.; Battarbee, R.; Yang, X.D.; Yang, H.D.; Liu, E.F. Tracking eutrophication in Taihu Lake using the diatom record: Potential and problems. *J. Paleolimnol.* 2008, 40, 413–429. [CrossRef]

32. Zhu, W.; Tan, Y.Q.; Wang, R.C.; Feng, G.Y.; Chen, H.; Liu, Y.P.; Li, M. The trend of water quality variation and analysis in typical area of lake taihu, 2010–2017. *J. Lake Sci.* 2018, 30, 296–305.

33. Yang, D.; Chen, W.; Jiang, J.; Ji, J.; Cao, W. Effects of algal bloom on N, P and K contents in Meiliang Bay of Taihu Lake. *Water Resour. Prot.* 2009, 25, 48–51.

34. Wang, J.; Jiang, X.; Zheng, B.; Chen, C.; Kang, X.; Zhang, C.; Song, Z.; Wang, K.; Wang, W.; Wang, S. Effect of algal bloom on phosphorus exchange at the sediment–water interface in Meiliang Bay of Taihu Lake, China. *Environ. Earth Sci.* 2016, 75, 1–9. [CrossRef]

35. Mao, X.W.; Xu, F.; Xu, B.; Gao, Y. Changes of water quality and eutrophication in Taihu Lake. *Water Resour. Prot.* 2009, 25, 48–51.

36. Sommer, S.; Türk, M.; Kriwanek, S.; Pfannkuche, O. Gas exchange system for extended in situ benthic chamber flux measurements under controlled oxygen conditions: First application—sea bed methane emission measurements at Captain Arutyunov mud volcano. *Limnol. Oceanogr. Methods* 2008, 6, 23–33. [CrossRef]

37. Kim, D.H.; Matsuda, O.; Yamamoto, T. Nitrification, denitrification and nitrate reduction rates in the sediment of Hiroshima Bay, Japan. *J. Oceanogr.* 1997, 53, 317–324.

38. Hall, G.H. Measurement of nitrification rates in lake sediments: Comparison of the nitrification inhibitors nitrapyrin and allylthiourea. *Microbial. Ecol.* 1984, 10, 25–30. [CrossRef] [PubMed]

39. Barnes, J.; Owens, N.J.P. Denitrification and nitrous oxide concentrations in the Humber estuary, UK, and adjacent coastal zones. *Mar. Pollut. Bull.* 1999, 37, 247–260. [CrossRef]

40. Xiao, M.; Bao, F.Y.; Wang, S.; Cui, F. Water quality assessment of the Huaihe River segment of Bengbu (China) using multivariate statistical techniques. *Water Resour.* 2016, 43, 166–176. [CrossRef]

41. Johnson, K.M.; Hughes, J.E.; Donaghy, P.L.; Sieburth, J.M. Bottle-calibration static head space method for the determination of methane dissolved in seawater. *Anal. Chem.* 1990, 62, 2408–2412. [CrossRef]

42. Weiss, R.F.; Price, B.A. Nitrous oxide solubility in water and seawater. *J. Environ. Eng.* 1980, 106, 247–257. [CrossRef]

43. Yang, J.; Zhang, G.; Zhao, Y.; Li, P. Production and emission of nitrous oxide from the estuarine tidal flat around Jiaozhou bay and their influencing factors. *Acta Sci. Circumst.* 2011, 31, 2723–2732.

44. Lim, B.; Ki, B.; Choi, J.H. Evaluation of nutrient release from sediments of artificial lake. *J. Environ. Eng.* 2011, 137, 347–354. [CrossRef]

45. Marier, M.; Schack-Kirchner, H. Using the gradient method to determine soil gas flux: A review. *Agric. For Meteorol.* 2014, 197, 256–257. [CrossRef]

46. Dai, X.; Qian, P.; Liang, Y.E.; Song, T. Changes in nitrogen and phosphorus concentrations in Lake Taihu, 1985–2015. *J. Lake Sci.* 2016, 28, 935–943.

47. Yu, J.; Fan, C.X.; Zhong, J.C.; Zhang, Y.L.; Wang, C.H.; Zhang, L. Evaluation of in situ simulated dredging to reduce internal nitrogen flux across the sediment-water interface in Lake Taihu, China. *Environ. Pollut.* 2016, 214, 866–877. [CrossRef] [PubMed]

48. Liu, X.L.; Liu, C.Q.; Li, S.L.; Wang, F.S.; Wang, B.L.; Wang, Z.L. Spatiotemporal variations of nitrous oxide (N$_2$O) emissions from two reservoirs in SW China. *Atmos. Environ.* 2011, 45, 5458–5468. [CrossRef]

49. Smolenski, R.L.; Beaulieu, J.; Townsendsmall, A.; Nietch, C. Spatial and temporal variations in greenhouse gas emissions from an agricultural reservoir. In Proceedings of the AGU Fall Meeting, San Francisco, CA, USA, 3–7 December 2012.

50. Wang, H.; Wang, W.; Yin, C.; Wang, Y.; Lu, J. Littoral zones as the “hotspots” of nitrous oxide (N$_2$O) emission in a hyper-eutrophic lake in China. *Atmos. Environ.* 2006, 40, 5522–5527.
51. Jäntti, H.; Stange, F.; Leskinen, E.; Hietanen, S. Seasonal variation in nitrification and nitrate-reduction pathways in coastal sediments in the Gulf of Finland, Baltic Sea. *Aquat. Microb. Ecol.* 2011, 63, 171–181. [CrossRef]

52. Starry, O.S.; Valett, H.M.; Schreiber, M.E. Nitrification rates in a headwater stream: Influences of seasonal variation in C and N supply. *Freshw. Sci.* 2005, 24, 753–768. [CrossRef]

53. Osborne, B.B.; Baron, J.S.; Wallenstein, M.D. Moisture and temperature controls on nitrification differ among ammonia oxidizer communities from three alpine soil habitats. *Front. Earth Sci.* 2016, 10, 1–12. [CrossRef]

54. Myrstener, M.; Jonsson, A.; Bergström, A.K. The effects of temperature and resource availability on denitrification and relative N$_2$O production in boreal lake sediments. *J. Environ. Sci.* 2016, 47, 82–90. [CrossRef][PubMed]

55. Luijn, F.V.; Boers, P.C.M.; Lijklema, L. Comparison of denitrification rates in lake sediments obtained by the N$_2$ flux method, the $^{15}$N isotope pairing technique and the mass balance approach. *Water Res.* 1996, 30, 893–900. [CrossRef]

56. Pfenning, K.S.; McMahon, P.B. Effect of nitrate, organic carbon, and temperature on potential denitrification rates in nitrate-rich riverbed sediments. *J. Hydrol.* 1997, 187, 283–295. [CrossRef]

57. Zhong, J.; Fan, C.; Liu, G.; Lei, Z.; Shang, J.; Gu, X. Seasonal variation of potential denitrification rates of surface sediment from Meiliang Bay, Taihu Lake, China. *J. Environ. Sci.* 2010, 22, 961–967. [CrossRef]

58. Li, Y.; Wang, S.; Zhang, W.; Yuan, J.; Xu, C.; Li, Y. Potential drivers of the level and distribution of nitrogen in the hyporheic zone of Lake Taihu, China. *Water* 2017, 9, 544. [CrossRef]

59. Zhen, S.; Zhu, W. Analysis of isotope tracing of domestic sewage sources in Taihu Lake—A case study of Meiliang Bay and Gonghu Bay. *Ecol. Indic.* 2016, 66, 113–120. [CrossRef]

60. Hasegawa, T.; Okino, T. Seasonal variation of denitrification rate in Lake Suwa sediment. *Limnol* 2004, 5, 33–39. [CrossRef]

61. Herbert, R.A. Nitrogen cycling in coastal marine ecosystems. *FEMS Microbiol. Rev.* 1999, 23, 563–590. [CrossRef][PubMed]

62. Yang, L.; Lin, F.; Xu, Z.; Zhang, M.; Gao, Y. Effect of temperature on the activities of microorganism and the pollutants release in the bioremediation of the sediment. *Envir. Pollut. Cont.* 2007, 95, 303–306.

63. Rütting, T.; Clough, T.J.; Müller, C.; Lieffering, M.; Newton, P.C.D. Ten years of elevated atmospheric carbon dioxide alters soil nitrogen transformations in a sheep-grazed pasture. *Glob. Chang. Biol.* 2010, 16, 2530–2542.

64. Svensson, J.M. Emission of N$_2$O, nitrification and denitrification in a eutrophic lake sediment bioturbated by Chironomus plumosus. *Aquat. Microb. Ecol.* 1998, 14, 289–299. [CrossRef]

65. Meyer, R.L.; Allen, D.E.; Schmidt, S. Nitrification and denitrification as sources of sediment nitrous oxide production: A microsensor approach. *Mar. Chem.* 2008, 110, 68–76. [CrossRef][PubMed]

66. Bouwman, A.F.; Beusen, A.H.W.; Griffioen, J.; Groenigen, J.W.V.; Hefting, M.M.; Oenema, O.; Puijenbroek, P.J.T.M.V.; Seitzinger, S.; Slomp, C.P.; Stehfest, E. Global trends and uncertainties in terrestrial denitrification and N$_2$O emissions. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 2013, 368, 91–97. [CrossRef][PubMed]

67. Muñoz-Hincapié, M.; Morell, J.M.; Corredor, J.E. Increase of nitrous oxide flux to the atmosphere upon nitrogen addition to red mangroves sediments. *Mar. Pollut. Bull.* 2002, 44, 992–996. [CrossRef]

68. Seitzinger, S.P.; Nielsen, L.P.; Caffrey, J.; Christensen, P.B. Denitrification measurements in aquatic sediments: A comparison of three methods. *Biogeochemistry* 1993, 23, 147–167. [CrossRef]

69. Bollmann, A.; Conrad, R. Acetylene blockage technique leads to underestimation of denitrification rates in oxic soils due to scavenging of intermediate nitric oxide. *Soil Biol. Biochem.* 1997, 29, 1067–1077. [CrossRef]
72. Li, Y.P.; Acharya, K.; Stone, M.C.; Yu, Z.B.; Young, M.H.; Shafer, D.S.; Zhu, J.T.; Gray, K.; Stone, A.; Fan, L.; et al. Spatiotemporal patterns in nutrient loads, nutrient concentrations, and algal biomass in Lake Taihu, China. *Lake Reserv. Manag.* 2011, 27, 298–309. [CrossRef]

73. Zhou, Q.C.; Zhang, Y.L.; Lin, D.W.; Shan, K.; Luo, Y.; Zhao, L.; Tan, Z.W.; Song, L.R. The relationships of meteorological factors and nutrient levels with phytoplankton biomass in a shallow eutrophic lake dominated by cyanobacteria, lake Dianchi from 1991 to 2013. *Environ. Sci. Pollut. Res.* 2016, 23, 15616–15626. [CrossRef] [PubMed]

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