Nonlinear pattern and algal dual-impact in N_2O emission with increasing trophic levels in shallow lakes

Yiwen Zhou[a,c,1], Xiaoguang Xu[b,1], Kang Song[a,d,*], Senbati Yeerken[a,d], Ming Deng[a], Lu Li[a], Shohei Riya[c], Qilin Wang[d], Akihiko Terada[c]

[a] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China

[b] School of Environment, Nanjing Normal University, Nanjing 210023, China

[c] Department of Chemical Engineering, Tokyo University of Agriculture and Technology, Tokyo 184-8588, Japan

[d] University of Chinese Academy of Sciences, Beijing, China

[e] Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, NSW 2007, Australia

[1] *Both authors contributed equally.*

**Corresponding author:*

No.7 Donghu South Road, Wuhan 430072, China; Email: sk@ihb.ac.cn (K. Song)

Abstract: Shallow lakes are considered important contributors to emissions of nitrous oxide (N_2O), a powerful greenhouse gas, in aquatic ecosystems. There is a large degree of uncertainty regarding the relationship between N_2O emissions and the progress of lake eutrophication, and the mechanisms underlying N_2O emissions are poorly understood. Here, N_2O emission fluxes and environmental variables in different lakes along a trophic state gradient in the Yangtze River basin were studied. N_2O emission fluxes were –1.0–53.0 μg m^{-2} h^{-1} and 0.4–102.9 μg m^{-2} h^{-1} in summer and winter, respectively, indicating that there was marked variation in N_2O emissions among lakes of different trophic state. The non-linear exponential model explained differences in N_2O emission fluxes by the degree of eutrophication ($p < 0.01$). In addition, seasonal variation in N_2O emission fluxes was higher in winter than in summer. TN and chl-*a* both predicted 86% of the N_2O emission fluxes in shallow lakes. The predicted N_2O emission fluxes based on the IPCC EF_{5r} overestimated the observed fluxes, particularly those in hyper-eutrophic lakes. These findings demonstrated that nutrient-rich conditions and algal accumulation are key factors determining N_2O emission fluxes in shallow lakes. Furthermore, this study also revealed that temperature and algae accumulation-decomposition determine an N_2O emission flux in an intricate manner. A low temperature, *i.e.*, winter, limits algae growth and low oxygen consumption for algae decomposition. The environment leaves a high dissolved oxygen concentration, slowing down N_2O consumption as the final step of denitrification. Such cascading events explained the higher N_2O emission fluxes from shallow lakes in winter

compared with summer. This trend was amplified in hyper-eutrophic shallow lakes after algal disappearance. Collectively, algal accumulation played a dual role in stimulating and impeding N_2O emissions, especially in hyper-eutrophic lakes. This study expands our knowledge of N_2O emissions from shallow lakes in which eutrophication is underway.

Keywords: Nitrous oxide, Biogeographic scale, Eutrophication, Functional gene, Algal accumulation

1 Introduction

Nitrous oxide (N_2O) is an ozone-depleting and highly potent greenhouse gas (GHG) with a long half-life that contributes to global warming, in addition to carbon dioxide (CO_2) and methane (CH_4), in the stratosphere (Ravishankara et al., 2009). Atmospheric N_2O has increased by 20% from 1750 to 2018 and is steadily increasing at a rate of 0.2% per year (Tian et al., 2020). The IPCC reported that approximately 10% of anthropogenic N_2O sources are derived from freshwater and coastal marine systems (IPCC, 2013). Given that inland freshwater lakes are recipients of nutrients transported from terrestrial ecosystems to trigger uncertainty of GHG emissions (Wang et al., 2009; Zhou et al., 2020a), they function as N_2O hot spots where the high turnover of nitrogen (N) compounds. Hence, the N_2O emissions from aquatic ecosystems have received considerable attention (Beaulieu et al., 2011; Kortelainen et al., 2020). Although multiple factors such as nutrient composition, eutrophication, and temperature likely regulate N_2O

61 emissions from freshwater lakes at regional and global scales, the dominant factors
62 affecting N_2O emissions in freshwater lakes remain poorly understood (Kortelainen et al.,
63 2020; Li et al., 2018; Yan et al., 2017). There is also a need to evaluate and quantify the
64 N_2O emissions of freshwater lake ecosystems given that they are globally significant
65 sources of N_2O (Lauerwald et al., 2019). Generally, understanding the mechanisms
66 underlying variation in N_2O emissions from freshwater lakes can aid the development of
67 policies to address global warming.

68 N_2O is mainly produced by a by-product from nitrification and an intermediate from
69 denitrification (Wenk et al., 2016). Of these multiple sources, denitrification is thought to
70 be a main source of N_2O in aquatic ecosystems (Beaulieu et al., 2011; Li et al., 2019a;
71 Salk and Ostrom, 2019). N_2O reduction, the final step of denitrification ($NO_3^- \rightarrow NO_2^-$
72 $\rightarrow NO \rightarrow N_2O \rightarrow N_2$), is catalyzed by N_2O reductase, which is encoded by the *nos*Z gene
73 (Yoon et al., 2016). Denitrification plays a critical role in determining N_2O emission
74 fluxes, including whether aquatic ecosystems are N_2O sources or sinks. N_2O production
75 is regulated by microbial community structure in aquatic ecosystems (Zhao et al., 2018;
76 Zhao et al., 2019). Microbial community composition affects the amount of N_2O
77 emissions, as some bacteria lack *nos*Z and nitrite reductase genes, which significantly
78 contribute to N_2O consumption and production in natural ecosystems, respectively
79 (Domeignoz-Horta et al., 2016). Among several environmental factors, nutrients, redox
80 conditions, and temperature determine the microbial guilds involved in N_2O production

and consumption (Hinshaw and Dahlgren, 2013; Xiao et al., 2019). Seasonal changes involve multiple environmental fluctuations that affect lacustrine microbial community structure (Song et al., 2012) and lead to uncertainty in the magnitude of N_2O emissions. N conversion rates and eutrophication progress are crucial for regulating final N forms (N_2 or N_2O) in aquatic ecosystems (Jiang et al., 2020; Salk and Ostrom, 2019; Zhu et al., 2020). Such N conversions are dynamic and dependent on eutrophication progress (Li et al., 2018; Liikanen et al., 2003). There is thus a need to understand the microbial processes that regulate N_2O sources or sinks in lakes of different trophic state and determine spatial heterogeneity in N_2O emissions.

Shallow lakes receive massive amounts of nutrients from anthropogenic activities, which potentially lead to changes in lake trophic state (Zhou et al., 2020a; Zhou et al., 2019). GHG emissions from eutrophic shallow lakes have also been surveyed, and this work has shed light on differences in N_2O emission fluxes among shallow lakes of different trophic state. In these surveys, the commonly used default emission factor (EF_{5r}) by the IPCC has been used (Maavara et al., 2019), which results in either an underestimation or overestimation of the N_2O budgets in freshwater ecosystems of different trophic state (Zhang et al., 2020). For example, Xiao et al.(2019) indicated that the mean N_2O emission fluxes in the East and West zones of Lake Taihu (eutrophic and oligotrophic, respectively) were substantially different, which is partially caused by N flowing to the lake. In addition, N loadings regulate the distribution of denitrifying

bacteria, which is often indicated by functional genes for N_2O production (*nirS* and *nirK*) and consumption (*nosZ*) (Huang et al., 2011; Zhao et al., 2018). High N flowing to eutrophic lakes increases algal growth, potentially enhancing N_2O emissions and N turnover rates (Yan et al., 2017; Zhu et al., 2020). However, the accumulation of algae alters the redox conditions favoring denitrification (Yan et al., 2017), and more severe anoxic conditions limit the supply of nitrogen oxides generated by nitrification required for denitrification (Zhu et al., 2020); these observations impede our understanding of the role of algae in N_2O emissions. Such correlations in eutrophic and hypereutrophic lakes have been extensively investigated (Lauerwald et al., 2019; Xiao et al., 2019); however, shallow lakes have been poorly studied. In particular, no studies have compared N_2O emission fluxes among lakes of different trophic state (Salk and Ostrom, 2019). The trophic state of lakes leads to uncertainty in N_2O emission estimates (Kortelainen et al., 2020), yet the relationship between N_2O emission fluxes and the trophic state of lakes is not entirely decoupled. Studies of the N_2O emissions of lacustrine trophic states on a biogeographic scale could help enhance our understanding of their potential to act as sources of N_2O emissions.

Approximately 0.9% of China is covered with lakes. There are a total of 2,693 lakes (> 1.0 km^2), about one-third of which are shallow lakes located in the middle and lower reaches of the Yangtze River basin (Ma et al., 2011). To enhance our understanding of the relationship between N_2O emissions and eutrophication in shallow lakes in the Yangtze

River basin, we characterized spatiotemporal variation in N_2O emission fluxes and its underlying mechanisms in 17 lakes along a trophic state gradient at a biogeographic scale. We constructed a model to predict the N_2O emission patterns in shallow lakes of different trophic state. The aims of this study were to (i) identify N_2O emission patterns in shallow lakes of different trophic state; (ii) characterize differences in the main microorganisms and functional genes for N_2O emissions in the sediments in shallow lakes of different trophic state; (iii) evaluate the relationship between environmental variables and N_2O emissions to reveal the main drivers of N_2O emissions; and (iv) elucidate the role of algae on N_2O emissions in shallow lakes. The results of this study enhance our ability to accurately predict N_2O emission patterns from shallow lakes.

2 Material and methods

2.1 Field survey

2.1.1 Lakes of different trophic state

This study designated 17 sampling shallow lakes (< 7 m deep) in the middle and lower reaches of the Yangtze River basin. Lakes were sampled in the winter (November) of 2017 and summer (August and September) of 2018. Lake Taihu, Lake Guchenghu, Lake Chaohu, and Lake Donghu were sampled in winter 2017. Because river inflow affects the environmental conditions of lakes (Zhou et al., 2019), all sampling sites were located more than 1 km away from the mouth of inflow rivers. Based on the trophic level

index (*TLI*) (see Supporting Materials for a description of how *TLI* was calculated), these lakes were classified into four trophic states: mesotrophic ($30 < TLI \leqslant 50$), eutrophic ($50 < TLI \leqslant 60$), middle-eutrophic ($60 < TLI \leqslant 70$), and hyper-eutrophic ($TLI > 70$) (Fig. 1) (Zhou et al., 2020b).

2.1.2 Heavy algae-accumulated and light algae-accumulated zones in Lake Taihu

Lake Taihu is a eutrophic lake that has experienced frequent and intensive cyanobacteria blooms since the 1980s (Qin et al., 2010). To characterize N_2O emission fluxes with or without algae accumulation, three typical zones (from west to east) in Lake Taihu, heavy algae-accumulated (Zone A and B), transitional (Zone C), and light algae-accumulated zones (Zone D), were studied (Fig. 1c). Sampling was conducted in summer (July) and winter (November) in 2019. The physicochemical parameters of the surface water and N_2O emission fluxes were investigated.

2.2 Sample collection and analysis

At each sampling event, vertical samples (*i.e.*, overlying water (20 cm below the water level), surface sediment (0–10 cm), and gas samples) were collected in triplicate. The *in situ* dissolved oxygen (DO), temperature, and pH were measured with DO, temperature, and pH probes (HQ3d, HACH, USA) on-site, respectively. To measure dissolved N_2 concentrations in summer, a water sample from a glass water sampler (1 L) was slowly drained from the bottom and transferred to a sample vial (12 mL) through a

159 silicone tube with minimal turbulence. The silicone tube was placed in the bottom of the
160 vial to avoid the ingress of atmospheric N_2. Next, 60 μL of saturated $HgCl_2$ solution (0.5%
161 v/v final concentration) was added to the sample vial to inhibit microbial activity. These
162 samples were stored in an ice cooler on-site and immediately transported to the laboratory
163 in a cooler at 4ºC. Water samples for chemical analyses, including total nitrogen (TN),
164 total phosphorus (TP), NO_3^--N, NH_4^+-N, dissolved organic carbon (DOC), and
165 chlorophyll-*a* (chl-*a*), were tested using previously described procedures (Zhou et al.,
166 2019). Briefly, TN and TP were measured using an ultraviolet spectrophotometry method
167 and an ammonium molybdate spectrophotometric method, respectively. NO_3^- and NH_4^+
168 concentrations were measured by a water flow analyzer (Auto Analyzer 3, Seal,
169 Germany), and the DOC concentration was determined using an elemental analyzer
170 (Flash EA 1112, CE Instruments, Italy). Chl-*a* was quantified by extraction in 95%
171 ethanol and measuring the absorbance at 630, 645, 663, and 750 nm using a UV-vis
172 spectrophotometer (UV-6100, Mapada, China).

173 **2.3 Tested and calculated N_2O emission fluxes**

174 N_2O emission flux was estimated by a floating static chamber (Cole et al., 2010;
175 Gålfalk et al., 2013). The headspace gas was collected between 11:00 and 14:00 using
176 three floating static chambers (size: 38.5 cm × 30.5 cm × 18.5 cm) following a previously
177 described procedure (Zhou et al., 2019). During each gas sampling event, six gas samples

were collected at 10-min intervals for 1 h via a static chamber. The gas chromatography (7890B Agilent) configuration described by Shaaban et al. (2018) was used to measure the N_2O concentration. The detailed methods for calculating N_2O emission fluxes are described in our previous study (Zhou et al., 2019). N_2O emission flux estimated by the floating static chamber method was calculated using Eq. (1):

$$F = \frac{V}{A} \times \frac{dC}{dt}, \quad (1)$$

where F is the N_2O emission flux (μg m^{-2} h^{-1}); V (m^3) and A (m^2) are the static chamber volume and surface area, respectively; and dC/dt is the time derivative of the N_2O concentration (μg m^{-3} h^{-1}).

2.4 Dissolved N_2 concentration and excess dissolved N_2 concentration

Dissolved N_2 was measured by a membrane inlet mass spectrometer system (MIMSS) with a probe inlet (HPR-40, Hiden Analytical Co.) using the N_2:Ar method described in a previous study (Chen et al., 2014). N_2:Ar ratios were calculated based on the quadrupole instrument signal (N_2 and Ar pressures at a detector) and calibrated using air-equilibrated water standards (Weiss, 1970). The dissolved N_2 concentrations of triplicate water samples were analyzed, and excess dissolved N_2 concentrations (ΔN_2) were calculated following previously described methods (Chen et al., 2014). ΔN_2 (μmol L^{-1}) was calculated using Eq. (2):

$$\Delta N_2 = N_{2\,(water)} - N_{2(eq)}, \quad (2)$$

where $N_{2(water)}$ is the dissolved N_2 concentration in water measured by MIMSS, and $N_{2(eq)}$ is the concentration expected if the water were in equilibrium with the atmosphere. Both were estimated following previously described methods (Weiss, 1970; Weiss and Price, 1980).

2.5 Prediction of N_2O emission fluxes based on the IPCC model

A predictive model was used to determine the N_2O emission factor (EF_{5r}) as recommended in the IPCC-2019 guidelines. The dissolved N_2O concentration (μg-N L^{-1}) was estimated using Eq. (3):

$$N_2O\text{-}N = NO_3^-\text{-}N \times EF_{5r}, \qquad (3)$$

where EF_{5r} is 0.26% according to the IPCC-2019 default value (IPCC, 2019), and NO_3^--N (μg-N L^{-1}) represents the concentration measured in a water column. N_2O emission fluxes (F', μg m^{-2} h^{-1}) were calculated by the dissolved N_2O concentration using the two-layer model of diffusive gas exchange, which is given as Eq. (4)

$$F' = k \times (C_w - C_{eq}), \qquad (4)$$

where C_w, is obtained from Eq. 3 and is the dissolved N_2O concentration in water estimated by the EF_{5r}; C_{eq} is the N_2O concentration in water that is in equilibrium with the atmosphere at the *in situ* air pressure and temperature; k is the gas transfer coefficient (m d^{-1}) and was normalized to the Schmidt number of 600, as described in the Supporting Materials (Cole and Caraco, 1998).

2.6 DNA extraction, high-throughput sequencing, and real-time qPCR analysis

Biomass for the sediment microbial community analysis was collected from lakes of different trophic state. DNA was extracted from the collected biomass using the DNA Isolation Kit (MOBIO, USA) per the manufacturer's instructions. The concentration and purity of DNA were measured using a microvolume UV-VIS spectrophotometer (NanoDrop^{TM} One^{C}, Thermo Fisher Scientific, USA), and the extracted DNA was stored at −20ºC before further analysis. 16S rRNA gene high-throughput sequencing was conducted using an Illumina MiSeq platform (Magigene Biotechnology Co. Ltd., Guangzhou, China). The primers used for high-throughput sequencing were modified 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) targeting the V3 and V4 hypervariable regions of both bacterial and archaeal 16S rRNA genes (Zhou et al., 2020b). Given that the *nirK* and *nirS* genes and the *nosZ* gene encode enzymes for N_2O production and consumption in denitrification, respectively (Zhao et al., 2018), the abundances of these three genes were analyzed. 16S rRNA gene abundances were quantified by real-time quantitative PCR (qPCR) with reported primer sets (Table S3). The PCR conditions for the amplification of *nirK, nirS*, and *nosZ* were described in a previous study (Chen et al., 2017).

2.7 Statistical analysis

Statistical analyses were conducted using SPSS 19.0 (SPSS Inc., Chicago, USA).

Significant differences among lakes were determined by one-way analysis of variance (ANOVA). The thresholds for statistically significant and highly statistically significant were $p < 0.05$ and $p < 0.01$ (two-tailed), respectively. The Kolmogorov-Smirnov test was conducted to determine if the data were normally distributed. The built-in exponential model of Origin 2019 software (OriginLab Inc., USA) was carried out to assess the relationship between N_2O emission fluxes and *TLI*.

3 Results

3.1 *In situ* N_2O emission fluxes in lakes of different trophic state

The *TLI* of the examined lakes ranged from 44.0 to 69.7 and from 43.3 to 76.7 in the summer and winter, respectively (Fig. S1). The hyper-eutrophic sampling sites were not included in the winter dataset. Among these examined lakes, the ranges of N_2O emission fluxes were –1.0–53.0 μg m^{-2} h^{-1} and 0.4–102.9 μg m^{-2} h^{-1} in summer and winter, respectively, indicating a high degree of variation in N_2O emission fluxes (Table S1 and Fig. 2). In addition, all examined lakes were N_2O sources, with the exception of mesotrophic lakes in summer. The mean N_2O emission fluxes of the middle-eutrophic lakes in winter (50.4 μg m^{-2} h^{-1}) were higher than those of the hyper-eutrophic (39.1 μg m^{-2} h^{-1}) and middle-eutrophic (7.4 μg m^{-2} h^{-1}) lakes in summer (Table S1). Overall, the N_2O emission fluxes gradually increased as *TLI* increased (Fig. 2a).

The lacustrine N_2O emission fluxes in both winter and summer were positively

correlated with *TLI* (Fig. 2a). The N_2O emission fluxes exponentially increased with the *TLI*s of the sampled lakes (adj. $R^2 = 0.85$, $p < 0.01$ in summer; adj. $R^2 = 0.63$, $p < 0.01$ in winter). There was a non-linear relationship between the net N_2O emission fluxes and *TLI* (adj. $R^2 = 0.36$, $p < 0.01$) (Fig. 2b). The N_2O emission fluxes increased as the *TLI* of lakes increased, and the increase was more pronounced in hypertrophic lakes. In addition, N_2O emission fluxes were predicted based on the EF_{5r} [Equation (3)]. There was a nonlinear exponential relationship between the predicted N_2O emission fluxes and *TLI* (adj. $R^2 = 0.80$, $p < 0.01$) (Fig. 2b). These fluxes were higher than the observed values, especially in the hyper-eutrophic lakes in summer (*TLI* > 70) (Fig. S2).

3.2 Dissolved N_2 concentration in shallow lakes

Mean dissolved N_2 concentrations across all lakes in summer ranged from 403.3 μmol L^{-1} to 443.8 μmol L^{-1} and exhibited an unimodal relationship with *TLI* (Fig. 3a). Among these examined lakes, the lowest dissolved N_2 concentration was observed in hyper-eutrophic lakes. There was a significant unimodal relationship between the dissolved N_2 concentration and *TLI* ($p < 0.001$). The excess dissolved N_2 (ΔN_2), which was obtained by subtracting dissolved N_2 from the saturated concentration, was consistently positive (8.1–16.1 μmol L^{-1}) in summer, indicating N_2 oversaturation. The trend of N_2 oversaturation as a function of *TLI* was consistent with dissolved N_2 (Fig. 3).

3.3 Relationship between environmental factors and N_2O emission fluxes

The Pearson correlations between N_2O emission fluxes and environmental parameters of the overlying water were analyzed in different seasons (Table S4). The N_2O emission fluxes were significantly and positively correlated with TN, NO_3^--N, and *TLI* ($p < 0.01$). The correlation between chl-*a* and N_2O emission fluxes varied seasonally, and the correlation was stronger in summer. ΔN_2 was negatively correlated with N_2O emission fluxes, TN, NO_3^--N, and chl-*a* in summer ($p < 0.05$). There was a negative correlation between DO concentrations and N_2O emission fluxes ($p < 0.05$) in summer, and this correlation was not observed in winter and over the entire year ($p > 0.05$) (Table S4). The overlying water DO was higher in winter (> 7 mg L^{-1}) than in summer (Fig. S1b). During the survey period, the temperature of the overlying water ranged from 9.5°C to 15.1°C and 27.1°C to 31.3°C in winter and summer, respectively (Fig. S1c). The temperature was negatively correlated with N_2O emission fluxes in both summer and winter ($p < 0.05$, Table S5). N_2O emission fluxes showed significant and positive linear correlations with TN (adj, $R^2 = 0.797$, $p < 0.01$ in winter; adj. $R^2 = 0.908$, $p < 0.01$ in summer), NO_3^--N (adj. $R^2 = 0.787$, $p < 0.01$ in winter; adj. $R^2 = 0.826$, $p < 0.01$ in summer), and NH_4^+-N (adj. $R^2 = 0.39$, $p = 0.039$ in winter; adj. $R^2 = 0.484$, $p = 0.01$ in summer) (Fig. 4). In addition, there was a significant negative linear correlation between *TLI* and the C:N ratio (adj. $R^2 = 0.649$, $p < 0.001$) (Fig. S3). There was a strong nonlinear correlation between N_2O emission fluxes and the C:N ratio (adj. $R^2 = 0.414$, $p < 0.001$) (Fig. S5).

A multiple stepwise regression model incorporating the physicochemical variables

293 of the overlying water was established for N_2O emission fluxes (Table 1). The results
294 showed that TN and TP concentrations can predict N_2O emission fluxes in summer (adj.
295 $R^2 = 0.94$, $p < 0.001$). TN ($t_1 = 10.80$) was more strongly positively correlated with N_2O
296 emission fluxes than TP ($t_2 = 2.93$), which indicated that TN was an important parameter
297 determining N_2O emission fluxes in summer. In addition, TN, chl-*a*, and NO_3^--N could
298 jointly predict N_2O emission fluxes (adj. $R^2 = 0.96$, $p < 0.001$) in winter, demonstrating
299 that both TN (6.11) and chl-*a* (–6.56) are important parameters determining N_2O emission
300 fluxes in winter. TN (12.83) and chl-*a* (–4.07) were important variables predicting N_2O
301 emission flux in lakes (adj. $R^2 = 0.86$, $p < 0.01$).

302 **3.4 Microbial community structure and denitrifier abundances in lakes of different**
303 **trophic state**

304 Relative abundances of microbes were obtained at the phylum level in summer and
305 winter (Fig. 5). Overall, the top 15 phyla made up more than 80% of microbial
306 communities in all lake sediments. The following six phyla accounted for over 60% of
307 the total population in summer: *Proteobacteria*, *Chloroflexi*, *Bacteroidetes*,
308 *Acidobacteria, Verrucomicrobia,* and *Planctomycetes*; those in the winter were
309 *Proteobacteria, Bacteroidetes, Acidobacteria, Nitrospirae, Planctomycetes,* and
310 *Verrucomicrobia.* The most abundant phylum was *Proteobacteria*, which had relative
311 abundances in hyper-eutrophic, middle-eutrophic, eutrophic, and mesotrophic lakes of

312 30.6–44.2%, 32.3–42.4%, 31.8–39.9%, and 32.0–46.4%, respectively. *Proteobacteria*
313 (26.4–43.4%) was also common in all lakes in winter. Among the six dominant phyla, the
314 relative abundances of *Chloroflexi* were 2.8–5.7% in winter and 4.7–15.9% in summer;
315 the relative abundances of *Nitrospirae* were 2.3–9.7% in winter and 0.1–4.1% in summer.
316 The *nirS* gene abundances in winter (0.66×10^7–3.67×10^7 copies g^{-1}-sediment)
317 were lower than those in summer (2.70×10^7–7.62×10^8 copies g^{-1}-sediment) (Fig. S5).
318 By contrast, no significant difference was observed for *nirK* gene abundances in winter
319 (0.36×10^7–2.29×10^7 copies g^{-1}-sediment) and summer (0.06×10^7–2.06×10^7 copies
320 g^{-1}-sediment). The *nirS*/*nirK* ratio was greater than 1, indicating that the *nirS*-type
321 denitrifiers were consistently more abundant than *nirK*-type denitrifiers in these shallow
322 lakes (Fig. 6a). The ratios were 9–48 times higher in summer than in winter. In addition,
323 the *nirS*/*nirK* ratio increased as *TLI* increased in summer and winter ($p < 0.05$). *nirS*/*nirK*
324 and N_2O emission fluxes were also positively correlated (Fig. S6a). The abundance of
325 *nosZ*, which encodes an enzyme for N_2O consumption, varied from 0.31×10^7 copies g^{-1}-
326 sediment to 2.88×10^7 copies g^{-1}-sediment in winter, which was lower than that in
327 summer (0.53×10^7–7.02×10^7 copies g^{-1}-sediment) (Fig. S5). Variation in the ratios of
328 (*nirK* + *nirS*)/*nosZ* was lower among the shallow lakes in winter (0.8–9.5) than in summer
329 (2.9–12.3) (Fig. 6b). The ratio of (*nirK* + *nirS*)/*nosZ* was positively correlated with *TLI*
330 ($p = 0.022$) and N_2O emission flux (Fig. S6b). Further analysis revealed the relationship
331 between the spatial distribution of these denitrifying genes and multiple environmental

factors (Fig. 7). A redundancy analysis (RDA) showed that the first two axes explained 60.38% of the variation in the denitrifying genes. The samples of the examined lakes were well separated among the different seasons. The RDA between the denitrifying gene abundances and environmental parameters indicated that temperature was an important factor affecting gene abundances. Among the tested parameters, *nirS* and *nosZ* abundances were sensitive to temperature compared with *nirK*. The dissimilarity in the abundance of denitrifying genes in lakes of different trophic state was greater in summer than in winter. The abundance of *nirS* was positively correlated with *TLI*, and the abundance of *nosZ* was marginally correlated with *TLI*.

3.5 Characterization of N_2O emissions in the heavy algae-accumulated and light algae-accumulated zones in Lake Taihu

The N loading and chl-*a* concentrations were higher in the heavy algae-accumulated zones (Zones A and B) than in the light algae-accumulated zone (Zone D) (Table S2). The N_2O emissions fluxes were characterized in summer and winter in the two typical zones in Lake Taihu. The N_2O emission fluxes were significantly higher in the heavy algae-accumulated zones (Zones A and B) and transitional zone (Zone C) than in the light algae-accumulated zone (Zone D). The N_2O emission fluxes were location-dependent and varied from 42.16–136.63 $\mu g\ m^{-2}\ h^{-1}$ in the heavy algae-accumulated zones (Zones A and B), 21.35–31.89 $\mu g\ m^{-2}\ h^{-1}$ in the transitional zone (Zone C), and 3.7–4.71 $\mu g\ m^{-2}\ h^{-1}$ in

the light algae-accumulated zone (Zone D) (Fig. 8). These fluxes in the algae-accumulated zones were significantly different in summer and winter ($p < 0.05$), and differences were not significant in Zone C ($p = 0.076$) and Zone D ($p = 0.677$). There was an exponential relationship between *TLI* and N_2O emission fluxes (Fig. S7) (adj. $R^2 = 0.55$, $p < 0.05$).

4 Discussion

4.1 Nonlinear N_2O emission patterns

Shallow lakes are potential sources of N_2O emissions and have been extensively studied (Kortelainen et al., 2020; Lauerwald et al., 2019; McCrackin and Elser, 2011). Previous studies have documented variation in N_2O emissions across lakes of different trophic state (Kortelainen et al., 2020; Salk and Ostrom, 2019); nevertheless, predicting N_2O emission fluxes from shallow lakes remains a challenge. This study showed that the net N_2O emission fluxes in lakes located in the Yangtze River basin, which spans 1000 km, displayed spatial and temporal heterogeneity determined by lake trophic state (Fig. 2). Consistent with previous studies (Salk and Ostrom, 2019; Xiao et al., 2019; Zhou et al., 2020a), these findings indicate that eutrophic lakes in the Yangtze River basin are sources of N_2O emissions. The patterns of N_2O emission fluxes in the shallow lakes were not completely consistent with the results of previous studies and depended on eutrophication progress. The N_2O emission fluxes in the eutrophic and middle-eutrophic

370 lakes (Table S1) were similar to the global median value of N_2O emission flux in lakes,
371 whereas the mean N_2O emission flux in the hyper-eutrophic lakes was 8.7–11.2 times
372 higher than the global median value (Hu et al., 2016). Shallow lakes in a mesotrophic
373 state in summer were N_2O sinks (Fig. 2 and Table S1); these findings expand our
374 knowledge regarding the prerequisites for freshwater lakes to act as either N_2O sinks or
375 sources (Lauerwald et al., 2019). Our study underscores the significance of lake trophic
376 state in determining N_2O emission fluxes, which is supported by the correlation between
377 net N_2O emission flux and trophic state (Fig. 2a). Whether lakes of different trophic state
378 act as N_2O sources or sinks can be predicted based on this correlation.

379 The major contribution of this study is the exponential model based on *TLI*, which
380 could provide a robust means for quantifying lake trophic state; this model can be used
381 to predict the N_2O emissions from lakes. This model represents an improvement over
382 previous approaches for estimating N_2O emission fluxes because previous approaches do
383 not consider differences in lake trophic state (Lauerwald et al., 2019). This model can be
384 used to assess N_2O emission fluxes in shallow lakes of different trophic state. The N_2O
385 emission fluxes and *TLI* were well fitted in summer (adj. $R^2 = 0.85$) and winter (adj. R^2
386 $= 0.63$). However, the coefficient of determination was low (adj. $R^2 = 0.36$) when all data
387 (*i.e.*, summer and winter) were incorporated into the model, which is likely explained by
388 the large differences in N_2O emission fluxes in winter and summer (Fig. 2). Therefore,
389 seasonal differences in N_2O emission fluxes in shallow lakes should also receive

390 consideration (Kortelainen et al., 2019; Miao et al., 2020). Given the limited data on
391 seasonal differences and the limited number of shallow lakes investigated, more data on
392 N_2O emission fluxes are required to verify the credibility of the model.

393 **4.2 Potential drivers of N_2O emissions**

394 Our results revealed that reactive N accumulation plays a major role in regulating
395 lake trophic state levels and the biological N cycle and promotes N_2O emissions via
396 denitrification in shallow lakes. Degradation and metabolism were similar among shallow
397 lakes despite variation in trophic state. *Proteobacteria* was identified as a predominant
398 phylum based on the 16S rRNA gene analysis, and it was commonly detected in lakes of
399 different trophic state (Fig. 5) (Li et al., 2019b); *Proteobacteria* might potentially
400 contribute to degradation and metabolism (Huang et al., 2019). Previous studies have
401 indicated that copiotrophic groups such as *Proteobacteria* and *Bacteroidetes* with high
402 growth rates tend to thrive in nutrient-rich conditions (Fierer et al., 2012). The overlying
403 water and sediments likely stored abundant nutrients that could be used by
404 microorganisms in shallow lakes. In addition, nutrient abundance is an important factor
405 affecting the microorganisms responsible for N conversion (Saarenheimo et al., 2015;
406 Zhang et al., 2019). The denitrifying genes varied greatly among the different trophic
407 lakes and seasons (Figs. 6 and S5). These results suggest that denitrification is an
408 important source of N_2O emissions in shallow eutrophic lakes, which is consistent with

409 the results of previous studies (Beaulieu et al., 2011; Zhang et al., 2020). This is also
410 confirmed by the stronger correlation of N_2O emission flux with NO_3^- than with NH_4^+
411 (Fig. 4) and the negative correlation between DO and N_2O emission fluxes (Table S4).
412 The significant relationship between *TLI* and (*nirK* + *nirS*)/*nosZ* (p = 0.022), which
413 indicates the relative abundance of N_2O producers relative to N_2O consumers, suggests
414 that N_2O production may be greater than N_2O consumption in hyper-eutrophic lakes (Fig.
415 6b) (Zhao et al., 2018). Higher net N_2O emission fluxes were observed in hyper-eutrophic
416 lakes because of their higher nutrient availability and (*nirK* + *nirS*)/*nosZ* (Kortelainen et
417 al., 2020). These findings might explain the close relationship between lake trophic state
418 and N_2O emission flux.

419 N availability drives eutrophication, and the subsequent accumulation of algae alters
420 the redox conditions favoring denitrification (Yan et al., 2017; Zhu et al., 2020) and
421 increases N_2O emissions in shallow lakes. In freshwater ecosystems, N loadings
422 significantly contribute to N_2O emissions via denitrification (Kortelainen et al., 2020;
423 Mulholland et al., 2008), which explains the high N_2O emission fluxes observed in
424 eutrophic lakes when N loading was high (Figs. 2 and 4). A stepwise linear regression
425 model indicated that TN and chl-*a* (algal density) are important parameters explaining
426 N_2O emissions (Table 1). Following algal accumulation, algal decay alters redox
427 conditions and releases organic matter *in-situ* (Yan et al., 2017; Zhu et al., 2020).
428 Moreover, algal blooms result in low DO concentrations and the accumulation of organic

429 matter in hyper-eutrophic lakes (Yan et al., 2017; Zhou et al., 2020b; Zhu et al., 2020).
430 For example, algal accumulation in Lake Taihu accounts for >50% of the organic matter
431 (Xu et al., 2019). A C:N ratio lower than 8 indicates that organic matter is mainly derived
432 from autochthonous inputs (Meyers, 1994; Yan et al., 2017). The negative correlation
433 between C:N ratio and *TLI* (Fig. S3) indicates that algal accumulation and decomposition
434 alter the physicochemical conditions in shallow lakes. Therefore, the contribution of algal
435 decomposition should receive increased consideration when exploring the relationship
436 between N_2O emission fluxes and chl-*a*. Our results indicated that N_2O emission fluxes
437 and the C:N ratio were negatively correlated ($p < 0.001$) (Fig. S5). Algal decomposition
438 results in oxygen consumption and thus a low DO concentration, which is favorable for
439 denitrification (Zhu et al., 2020). This observation is consistent with the negative
440 correlation between DO and chl-*a* (Tables S4 and S5). These findings might potentially
441 explain the roles of decomposed algae in stimulating N_2O emissions via consumed
442 oxygen in eutrophic shallow lakes.

443 Temperature is an important variable determining denitrifier abundance and
444 structure in both seasons (Figs. 6 and 7); the effect of temperature was also manifested by
445 the differences in N_2O emissions among seasons (Fig. 2). This pattern is similar to a
446 previous study of 87 boreal lakes in Finland showing that N_2O emissions peaked in winter
447 (Kortelainen et al., 2020). Among enzymes responsible for denitrification, N_2O reductase
448 is most sensitive to changes in temperature (Kortelainen et al., 2020; Veraart et al., 2011;

449 Zhou et al., 2020c). Our previous study indicated that *nosZ* in N_2O-reducing bacteria is
450 strongly related to temperature (Zhou et al., 2020c). In addition, N_2O reduction activity
451 is inhibited by oxygen exposure, which increases N_2O emissions (Song et al., 2019). Low
452 temperature increased the oxygen concentration (Fig. S1), and the relationship between
453 the ratio of *nirS* to *nirK* with *TLI* varied in winter (slope: 0.08) and summer (slope: 1.11)
454 ($p < 0.05$) (Fig. 6a). Given that the algae in lakes gradually declined in winter concomitant
455 with increasing DO concentrations (Fig. S1 and Table S1), N_2O emission fluxes in lakes
456 increased in winter (Fig. 2a) (Miao et al., 2020). In summer, algal decay further decreased
457 the oxygen concentration. Previous studies have shown that *nirK* only achieves high
458 abundances in conditionally oxygen-exposed environment (Huang et al., 2011), whereas
459 *nirS* genes have been more commonly detected in anoxic locations (Knapp et al., 2009).
460 The observation that the N_2O emission fluxes, *nirS*/*nirK*, and (*nirS*+*nirK*)/*nosZ* showed
461 more significant positive relationships in winter than in summer suggests that the N_2O
462 emission fluxes in summer were also affected by other factors (Fig. S6). The dramatic
463 difference between hyper-eutrophic lakes in winter and summer was in the frequency of
464 algal blooms in hyper-eutrophic lakes in summer. The algae regulating N_2O emission
465 fluxes in shallow lakes are discussed in the subsequent section. In sum, low temperature
466 associated with abundant N favored N_2O accumulation via control oxygen concentrations
467 and limit N_2O reduction activity.

468 **4.3 Dual impact of algae on N_2O emissions**

469 N_2O emissions were highest, and the chl-*a* concentration low, in Zone B in contrast
470 to Zone A in a heavy algae-accumulated zone of Lake Taihu (Fig. 8 and Table S2). This
471 result is inconsistent with the finding that algal accumulation, reflected by *TLI*, stimulated
472 N_2O emissions in a non-linear exponential manner in the other tested lakes (Fig. S7). In
473 heavy algae-accumulated zones, lower N_2O emissions, indicated by the high chl-*a*
474 concentration, likely stem from algal accumulation, which suppresses denitrification
475 activities caused by the decrease in N_2O production. Recently, Zhu et al. (2020) reported
476 that algal accumulation may inhibit denitrification during algal blooms in summer.
477 Consistent with this finding, a unimodal relationship between *TLI* and excess dissolved
478 N_2 (ΔN_2) in summer was observed (Fig. 3b), which indicated higher complete
479 denitrification (*i.e.*, including N_2O consumption) rates in the eutrophic lakes (Chen et al.,
480 2014; Wang et al., 2018). In summer, the highest gene abundances of *nirK*, *nirS*, and *nosZ*
481 were observed in eutrophic and middle-eutrophic lakes rather than in hyper-eutrophic
482 lakes (Fig. S5). Although more abundant N is available in hyper-eutrophic lakes, the
483 complete denitrification rates may be lower in hyper-eutrophic lakes than in eutrophic
484 lakes in summer. This pattern potentially stems from the abundance of algal biomass in
485 the hyper-eutrophic lakes, as release of algal debris eventually leads to reductive
486 conditions (Table S2). The decay of excess algal biomass could create favorable
487 conditions for denitrification where organic carbon is present under hypoxic or anoxic
488 conditions. However, hypoxia limits nitrification, which leads to a deficiency in the

supply of NO_3^- for denitrification (Small et al., 2014; Zhu et al., 2020). This effect is supported by previous work in zones with accumulated algae in Lake Taihu showing that the NH_4^+ concentration in sediments is two or three orders of magnitude higher than the NO_3^- concentration (Yan et al., 2019). Our results are also consistent with this observation (Table S2). In addition, chl-*a* (as a negative factor) and TN together predicted the N_2O emission fluxes (Table 1). Algal accumulation and decomposition create hypoxic conditions that limit nitrification by converting NH_4^+ into NO_3^-, eventually suppressing the ensuing denitrification. In addition, hypoxic conditions favor complete denitrifying bacteria for mitigating N_2O emissions. These results further explain why higher N_2O emission fluxes of shallow lakes were observed in winter rather than in summer (Figs. 2a and 8). Therefore, these evidences indicated that algal accumulation played a dual role in stimulating and impeding N_2O emissions, especially in hyper-eutrophic lakes.

4.4 Implications of eutrophication progress on N_2O emissions

In aquatic ecosystems, excessive N loadings drive eutrophication and promote N_2O emissions in water bodies (Zhao et al., 2015). Meanwhile, the nutrient overload induces algae growth in shallow lakes, forming an anoxic or microaerobic micro-environment favoring N_2O production by algae accumulation (Zhu et al., 2020). In this study, a nonlinear exponential increase in N_2O emission flux as a function of *TLI* in shallow lakes of different trophic state was observed (Fig. 2). Eutrophic lakes had high N_2O emission

508 fluxes, which is consistent with the relationship between lake trophic state and CH_4
509 emission fluxes (Zhou et al., 2020b). The common trends indicate that increases in GHG
510 emissions stem from eutrophication. The predicted N_2O emission fluxes based on the
511 IPCC EF_{5r} overestimated the observed fluxes but also exhibited a non-linear exponential
512 increase with *TLI* (Fig. 2b). Xiao et al. (2019) reported that the N_2O emission factor in
513 Lake Taihu was 0.18%, which is lower than the value of EF_{5r} based on the reported IPCC
514 value. The overestimation by the IPCC default value indicates the need to calibrate an
515 N_2O emission factor in shallow lakes depending on the eutrophic state. Pronounced
516 differences between the predicted and observed N_2O emission fluxes were observed in
517 hyper-eutrophic lakes where the abundance of algae impeded N_2O emissions. The high
518 abundance of algae in summer compared with winter indicates that the degree to which
519 N_2O emission flux was overestimated in summer was different from that in winter (Fig.
520 S2). Algal decomposition made the redox conditions favorable for denitrification but
521 unfavorable for nitrification because of an insufficient supply of NO_3^-. The retained
522 nitrogen is absorbed by the newly grown algae (Zhu et al., 2020). In non-limited N, algal
523 decay leads to low-oxygen conditions, which enhances denitrification and further
524 stimulates N_2O emissions. Therefore, algae should be considered a nitrogen "pool" that
525 maintains nitrogen in lakes. In such situations where algae pools N, lower N_2O emissions
526 stemming from the suppression of nitrification do not contribute to the reduction in global
527 N_2O emissions from lakes but potentially leads to substantial N_2O emissions from hyper-

528 eutrophic state lakes when conditions for nitrification are suitable. We suggest that
529 overestimation was possibly caused by the "dual role" of the algae because they help re-
530 regulate denitrification to mitigate N_2O emissions. Estimation of flux by the two-layer
531 model was one order of magnitude lower than that estimated by static chamber methods
532 (Duchemin et al., 1999). Given that the two different methods plausibly overestimated or
533 underestimated the N_2O emission fluxes in shallow lakes, an intensive survey of an N_2O
534 emission factor in hyper-eutrophic lakes will be conducted in a follow-up study.

535 **5 Conclusions**

536 We performed a series of field measurements and characterized the N_2O emissions
537 in shallow lakes of different trophic state in the Yangtze River basin. The results of this
538 study are detailed below.

539 • The N_2O emission fluxes of shallow lakes were most strongly affected by lake trophic
540 state, suggesting that estimation of N_2O emission fluxes should consider lake trophic state.

541 • The nonlinear model incorporating trophic state levels can describe the N_2O
542 emissions from a shallow lake.

543 • The predicted N_2O emission fluxes based on the IPCC EF_{5r} overestimated the
544 observed fluxes, particularly those in hyper-eutrophic lakes.

545 • Nutrient-rich conditions and algal accumulation were key factors determining N_2O
546 emission fluxes in shallow lakes, and algal accumulation played a dual role in stimulating

and impeding N_2O emissions, especially in hyper-eutrophic lakes.

- Changes in season accompanied the appearance and disappearance of algae and altered N_2O emission fluxes, especially in hyper-eutrophic lakes.

Conflicts of interest

The authors declare no competing interests.

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Table 1. Validity of the multiple stepwise regression model for N_2O emission fluxes incorporating variables of the overlying water

Season	Parameters	Equations	Variables	Adj. R^2	*P*	Significance level			
						F-test	T-test		
						F	t_1	t_2	t_3
Summer	N_2O	$N_2O = 14.33(TN) – 10.45$	TN	0.91	$p < 0.001$	158.86	12.60		
		$N_2O = 12.36(TN) + 27.99(TP) – 13.13$	TN, TP	0.94	$p < 0.001$	123.95	10.80	2.93	
Winter	N_2O	$N_2O = 21.04(TN) – 13.44$	TN	0.80	$p < 0.001$	56.11	7.49		
		$N_2O = 32.06(TN) – 1.79(\text{chl-}a) – 1.09$	TN, chl-*a*	0.90	$p < 0.001$	60.65	8.76	–3.62	
		$N_2O = 20.68(TN) – 2.04(\text{chl-}a) + 22.55(NO_3^-\text{-N}) + 3.13$	TN, chl-*a*, NO_3^--N	0.96	$p < 0.001$	112.69	6.11	–6.56	0.61
Summer + Winter	N_2O	$N_2O = 18.93(TN) – 13.80$	TN	0.79	$p < 0.001$	110.56	10.52		
		$N_2O = 23.45(TN) – 0.33(\text{chl-}a) – 12.03$	TN, chl-*a*	0.86	$p < 0.001$	74.08	12.83	–4.07	