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Nutrient enrichment—but not warming—increases nitrous oxide emissions from shallow lake mesocosms

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Abstract

Shallow lakes and ponds play a crucial role in the processing of carbon and other nutrients. However, many lakes and ponds worldwide are affected by climate change and nutrient pollution. How these pressures affect the emission of the greenhouse gas nitrous oxide (N_2O) is unclear. Warming and eutrophication are expected to increase the production and emission of N_2O in lakes and ponds, but changes in ecological structure and function may complicate these seemingly straightforward relationships. In this study, we used the world's longest running, mesocosm-based, freshwater climate change experiment to disentangle the effect of nutrient enrichment and warming on N_2O emissions. We gathered a large dataset on N_2O concentrations and ancillary variables, comprising three sampling campaigns between 2011 and 2020 and a total of 687 individual mesocosm measurements. Our results demonstrated that nutrient enrichment increased N_2O emissions, while warming (+2.5–4.0°C and +3.75–6.0°C) had no discernable effect. Our study indicates that curtailing nitrogen influxes into lakes and ponds is the most effective strategy to minimize N_2O emissions, and while warming may influence N_2O emissions, it does not appear to be a direct driver. These findings underscore the importance of prioritizing nitrogen mitigation efforts to curb N_2O emissions from shallow lakes and ponds.

Lakes cover 3.7% of the world's land surface, with shallow lakes and ponds being not only the most abundant types, but also having the largest areal extent of continental waters (Downing 2010; Verpoorter et al. 2014). Despite their limited size, shallow lakes and ponds play a crucial role in the regulation of global nutrient and carbon cycles, and they also support a rich biodiversity (Tranvik et al. 2009; Downing 2010; Meerhoff and Beklioğlu 2024).

Shallow lakes and ponds frequently suffer from eutrophication due to nutrient enrichment, often stemming from inadequate water treatment facilities or agricultural fertilizer use. Consequently, many waterbodies are in a poor ecological state. The ramifications of eutrophication are numerous and are frequently exacerbated by the effects of climate change (Moss et al. 2011; Meerhoff et al. 2022). For instance, it has been shown that eutrophication and warming, two of the multiple faces of global change, have synergistic effects on methane emissions in shallow lakes and ponds, leading to a disproportionate increase in the emission of this greenhouse gas (DelSontro et al. 2016; Davidson et al. 2018; Sepulveda-Jauregui et al. 2018). Although the impacts of eutrophication and warming on methane are relatively well established, their influence on the greenhouse gas nitrous oxide (N_2O) in lakes and ponds still requires further investigation (Velthuis and Veraart 2022). Global inland water N_2O emissions are estimated to contribute 10% to the

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global anthropogenic emissions and have steadily increased from 0.4 to 1.3 Tg N yr^{-1} during 1900–2010 primarily due to increasing fertilizer use (Wang et al. 2023). While natural lakes are considered a weak global source of N_2O, lakes and reservoirs impacted by eutrophication may exhibit high-N_2O emissions (Wang et al. 2006; Lauerwald et al. 2019; Maavara et al. 2019).

Nitrous oxide arises from nitrogen transformations in soils, sediments, and waters (Wrage et al. 2001; Baggs and Philippot 2011; Wrage-Mönnig et al. 2018). In lakes and ponds, microbial denitrification in sediment is generally considered the main N_2O production pathway, although other processes such as nitrification may also play a part (Jurado et al. 2017; Velthuis and Veraart 2022). Denitrification takes place under hypoxic to anoxic conditions and is the stepwise reduction of oxidized nitrogen compounds (nitrite or nitrate) dissolved in the water to gaseous forms (nitric oxide, N_2O, and dinitrogen) (Tiedje 1988; Wrage et al. 2001). Hence, denitrification is an efficient process to remove reactive nitrogen from the aquatic environment (Seitzinger et al. 2006). While it is expected that greater nitrogen input in the form of nitrate and the subsequent increase in denitrification will lead to an increase in N_2O production and emission (Silvennoinen et al. 2008; Baulch et al. 2011; Velthuis and Veraart 2022), several factors may complicate this seemingly straightforward relationship. In lakes and ponds, nutrient enrichment (i.e., eutrophication) leads to changes in ecological status, increasing the abundance of phytoplankton and altering the structure and functioning of plankton communities, with knock on effects on oxygen levels in the sediment, substrate availability, and it may alter N_2O production and emission (Smith et al. 1999). As with other biological processes that scale with temperature at the cellular level, denitrification and N_2O production in sediment are anticipated to be enhanced by increased temperature (Liikanen et al. 2002; Myrstener et al. 2016). Additionally, the decreased oxygen solubility at higher temperature may also promote denitrification and potentially N_2O production (Veraart et al. 2011). The complex and dynamic interactions governing nitrogen cycling in lakes create significant uncertainty regarding the combined impacts of warming and nutrient enrichment on N_2O emissions from shallow lakes.

In this study, the world's longest running, mesocosm-based, freshwater climate change experiment was used to disentangle the effects of warming and nutrient enrichment on N_2O emissions (using N_2O concentration as a proxy). The mesocosms are flow-through, groundwater-fed systems and thus representative of groundwater-fed shallow lakes or ponds worldwide. It was hypothesized that N_2O emissions are positively correlated with warming and nutrient enrichment.

Materials and methods

Experimental setup

The experiment was carried out at Aarhus University's lake mesocosm climate change facility located in Central Jutland, Lemming, Denmark (56°2448N, 9°5298E). These mesocosms are part of the world's longest running shallow lake mesocosms study initiated in August 2003 with the aim to investigate the effects of eutrophication and warming (*see* details in Liboriussen et al. 2005). Briefly, the experimental facility includes 24 outdoor and uncovered flow-through mesocosms (diameter 1.9 m, water depth 1 m, retention time about 2.5 months), which are continuously mixed and fed with groundwater (Liboriussen et al. 2005). At the start of the experiment in 2003, a 0.1-m layer of washed sand was placed at the bottom of each mesocosm with a 0.1-m layer of nutrient-rich sediment from a nearby freshwater pond (Liboriussen et al. 2005). The mesocosms are exposed to three warming treatments: unheated (Amb), heated treatment A2 (A2) according to the Intergovernmental Panel on Climate Change (IPCC)-predicted temperature increase (Houghton et al. 2001), and heated treatment A2 + 50% (A2+). The heated mesocosms are warmed up using heating elements placed about 10 cm above the sediment. The water temperature in the unheated mesocosms fluctuates with the air temperature, while the water temperature in the two heated treatments is continuously adjusted to be 2.5–4°C (A2) and 3.75–6°C (A2+) warmer than the unheated mesocosms depending on the season (Liboriussen et al. 2011). The seasonal differences between the temperature treatments are based on future climatic projections for Denmark downscaled to monthly resolution using 1961–1990 as reference period (Houghton et al. 2001). The three warming treatments are crossed with two nutrient treatments (low and high), yielding six treatments with four replicates. In the low-nutrient treatment, no nutrients are added, that is, the level is that of the inflowing water averaging 0.01 mg P L^{-1} and 2.30 mg N L^{-1}, corresponding to 0.15 mg P m^{-2} d^{-1} and 30.3 mg N m^{-2} d^{-1}, while in the high-nutrient treatment, nutrients are added weekly at loadings of 2.7 mg P m^{-2} d^{-1} and 108 mg N m^{-2} d^{-1} as sodium phosphate and calcium nitrate solutions, respectively. The purpose of these two nutrient levels is to mimic a clear water shallow lake and a turbid water shallow lake (Scheffer et al. 1993). Submerged macrophytes are generally abundant in the low-nutrient mesocosms, whereas the high-nutrient mesocosms are more turbid with abundant phytoplankton or filamentous algae.

Our dataset comprises three sampling campaigns: March 2011 to February 2012, April 2015 to April 2016, and May 2019 to February 2020, which means that the mesocosms had been consistently heated for 8, 12, and 16 yr, respectively, prior to the sampling campaigns. All campaigns included monthly or bimonthly sampling and totaled 687 individual mesocosm measurements for N_2O and ancillary variables. All samplings were carried out in the morning between 7 a.m. and 12 a.m. The data collected between March 2011 to February 2012 were published in Davidson et al. (2015).

Water chemistry and ancillary measurements

At each sampling round, depth-integrated water samples were collected from three randomly chosen locations within each mesocosm using a tube sampler. Total nitrogen, total phosphorus, and total iron were analyzed on unfiltered samples, after persulfate digestion. For dissolved nutrients (phosphate, nitrate, and ammonium) water samples were filtered through Whatman® glass microfiber filters grade GF/C (1.2 μm pore size) prior to analysis. For total nitrogen and nitrate, analyses were carried out with the cadmium reduction method on a Foss flow injection analyzer FIAStar 5000 (FOSS Analytical AB, Sweden), using the provided protocols (with Griess reagents). Spectrophotometric measurements were conducted for ammonium following the phenol–hypochlorite method, for total phosphorus and orthophosphate following the ascorbic acid method, for total iron following the hydroxylammonium chloride method, and for chlorophyll *a* (Chl *a*) following the ethanol extraction method, all in compliance with standard Danish/European methods. All spectrophotometric measurements were conducted using a Shimadzu UV-1800 spectrophotometer.

In the period May 2019 to February 2020, the water samples were collected on different dates than the greenhouse gas sampling. Linear interpolation between these dates was used to estimate the concentrations. There was no more than a week between the date of the N_2O sampling and the closest date of water chemistry sampling.

Water temperature at the time of sampling was recorded using PR electronics temperature sensors placed at 50 cm depth in the mesocosms. Dissolved oxygen was measured every 30 min using oxygen probes (OxyGuard®, two-wire probe, model 420) and a submersible pump (Sicce pumps, model: Micra) connected to the oxygen probe to create a continuous horizontal water flow over the membrane to ensure correct measurements. Water pH was also measured every 30 min using OxyGuard pH probes connected to pH Manta transmitters.

Daily (24 h) means of oxygen and pH are shown in the results. Oxygen probes were calibrated every 2nd week, and pH was calibrated monthly (*see* Supporting Information).

The abundance of macrophyte was recorded visually with a bathyscope in the mesocosms at every sampling event as plant volume inhabited (%).

Nitrous oxide concentrations, fluxes, and emission factors

On every sampling occasion, water samples were collected to determine the aqueous concentrations of N_2O using headspace equilibration (McAuliffe 1971) and gas chromatography. The methods varied slightly across the sampling campaigns but were all based on the same principle: a known volume of water was collected in a gas-tight container, and a headspace was introduced using inert gas (N_2) or ambient air. The sampling methods are described in detail in Davidson et al. (2015) and Audet et al. (2020). The headspace concentrations of N_2O were determined using a dual-inlet Agilent 7890 gas chromatograph system interfaced with a CTC CombiPal autosampler (Agilent, Nærum, Denmark) configured and calibrated with standard gases as described in detail by Petersen et al. (2012). The aqueous concentrations of N_2O were calculated from the headspace gas concentrations according to Henry's law and using Henry's constant corrected for temperature and salinity (Weiss and Price 1980). Because N_2O solubility is temperature dependent, all N_2O concentrations were standardized to a water temperature of 20°C to facilitate comparison across temperature treatments. This was done as follow:

$$C_{\mathrm{wat},20^\circ} = C_{\mathrm{wat},t^\circ} \times \frac{C_{\mathrm{eq},20^\circ}}{C_{\mathrm{eq},t^\circ}},$$

where $C_{\mathrm{wat},20^\circ}$ (g m^{-3}) is the concentration of N_2O dissolved in the water at 20°C, C_{wat,t° (g m^{-3}) is the concentration of N_2O dissolved in the water at the temperature of sampling t°, $C_{\mathrm{eq},20^\circ}$ (g m^{-3}) is the concentration of N_2O that the water would have at 20°C in equilibrium with the atmosphere assuming atmospheric concentrations of 0.330 ppm for N_2O or, if available, based on N_2O air concentrations measured on the sampling day. C_{eq,t° (g m^{-3}) is the concentration of N_2O that the water would have at the sampling temperature t° in equilibrium with the atmosphere.

The flux of N_2O between the water and the overlying atmosphere was estimated as:

$$f_{\mathrm{N_2O}} = k_{\mathrm{N_2O}}\left(C_{\mathrm{wat},t^\circ} - C_{\mathrm{eq},t^\circ}\right),$$

where $f_{\mathrm{N_2O}}$ (g m^{-2} h^{-1}) is the N_2O flux, $k_{\mathrm{N_2O}}$ (m h^{-1}) is the gas transfer velocity, and $C_{\mathrm{wat},t^\circ} - C_{\mathrm{eq},t^\circ}$ (g m^{-3}) is the gradient of concentration between the concentration of gas dissolved in the water (C_{wat,t°) and the concentration of gas that the water would have in equilibrium with the atmosphere (C_{eq,t°). Gas transfer velocities for the different gases were calculated based on direct measurements of the gas transfer velocity for O_2 in three mesocosms in December 2009 (Liboriussen et al. 2011) and corrected for the water temperature (Jähne et al. 1987). Only minor variation was found in the $k_{\mathrm{O_2}}$ estimates based on the recovery of artificial reduction in O_2 in three of the mesocosms (0.0080, 0.0086, and 0.0100 m h^{-1} at 20°C) (Liboriussen et al. 2011). Therefore, the same gas transfer velocity was applied to all mesocosms ($k_{\mathrm{O_2}} = 0.0088$ m h^{-1} at 20°C). Macrophyte abundance may affect water stratification and turbulence at the interface between water and atmosphere and thus alter the gas transfer velocity. However, because the mesocosms are all constantly mixed with automatic paddles, we assumed that between-mesocosm differences in gas transfer velocity were minor, and $k_{\mathrm{N_2O}}$ was calculated as follows:

$$k_{\mathrm{N_2O}} = k_{\mathrm{O_2}}\left(\frac{\mathrm{Sc}_{\mathrm{N_2O}}}{\mathrm{Sc}_{\mathrm{O_2}}}\right)^x,$$

where Sc_g is the Schmidt number for the gas g, that is, N_2O or O_2 (Wanninkhof 1992). We chose $x = -2/3$ as this factor is used for smooth liquid surfaces (Deacon 1981). Wind speed alters piston velocity; however, at low-wind speed ($< 3\ m\ s^{-1}$), the effect is negligible (Cole and Caraco 1998), which was the case in our study where the mesocosms were relatively well sheltered from wind.

The emission factors for N_2O were calculated as the ratio between N_2O fluxes and the nitrate input (via groundwater and, if any, nutrient addition to the mesocosms; Hergoualc'h et al. 2019).

Statistical analysis

All analyses relied on the use of linear mixed effect models fitted using the R package "nlme" (Pinheiro and Bates 2000; Pinheiro et al. 2022). A 1st analysis was performed to assess the relationship between the dissolved N_2O concentrations and the warming and nutrient treatments. In the 2nd analysis, relationships between the dissolved N_2O concentrations and environmental variables were explored. Because N_2O fluxes were estimated based on N_2O concentrations and constant values of k_{N_2O} in all mesocosms, we only ran analyses based on N_2O concentrations. Likelihood ratios were used to compare models, starting with a full model, including quadratic terms and interactions, sequentially dropping the least significant variable until all remaining covariables were significant (Zuur et al. 2009). A 3rd analysis was run to model the relationship between N_2O emission factors and the warming and nutrient treatments. To reduce heterogeneity of variance in the data, the dissolved N_2O concentrations were log transformed. This transformation helped reduce the heterogeneity of the data and allowed us to retain all data points for the statistical analysis, despite the presence of outliers. The model included a variance structure specified by "varIdent," which allowed varying levels of variability based on nutrient treatments. Incorporating an autocorrelation structure, such as "corAR1," did not yield a substantial improvement in the model's fits. The maximum likelihood method was utilized for parameter estimation. The assumptions of the modeling techniques used, chiefly independence and homogeneity, were checked visually by investigating residual patterns of the various models. The residual patterns of the models were also examined to mitigate any potential biases associated with the sampling periods. The statistical analysis was performed using the R software (R Development Core Team 2022).

Results

The N_2O concentration measured in the mesocosms across all treatments was 0.38 (0.31–0.50) $\mu g\ N\ L^{-1}$ (median (interquartile range)), ranging from 0.16 to 20.5 $\mu g\ N\ L^{-1}$ (Fig. 1a,b). At low-nutrient levels, the median was 0.33 (0.28–0.39) $\mu g\ N\ L^{-1}$, while at high-nutrient levels it was 0.47 (0.37–0.64) $\mu g\ N\ L^{-1}$ (Fig. 2a,b). In the unheated mesocosms, the median N_2O concentration was 0.37 (0.30–0.49) $\mu g\ N\ L^{-1}$, whereas it was 0.38 (0.31–0.47) and 0.39 (0.31–0.57) $\mu g\ N\ L^{-1}$ in the heated treatments A2 and A2+, respectively. Notably, in August 2019, one heated mesocosm at A2+ with high-nutrient levels recorded exceptionally high-N_2O concentration, exceeding the rest of the dataset by one order of magnitude.

Nitrous oxide emission across all treatments was 8.3 (0.77–29.4) $\mu g\ N\ m^{-2}\ d^{-1}$. At low-nutrient levels, it was 2.03 (0.29–14.5) $\mu g\ N\ m^{-2}\ d^{-1}$ and at high-nutrient levels, it was 21.2 (2.43–57.4) $\mu g\ N\ m^{-2}\ d^{-1}$. The mesocosms were generally a source of N_2O to the atmosphere. We found negative fluxes, which indicates undersaturation in 69 out of 687 individual measurements, that is, 10% of the measurements. Undersaturation was found irrespective of warming treatment (25, 23, and 21 observations at unheated, heated A2, and heated A2+, respectively) but occurred mostly in the low-nutrient treatments, in 65 out of 69 cases. Furthermore, N_2O undersaturation generally occurred at low-nitrate concentration and between late spring and early fall. Hence, in 74% of the cases of undersaturation, nitrate was below the detection limit. Furthermore, the records of N_2O undersaturation appeared between May and October in 74% of the cases.

Nitrous oxide concentration at high-nutrient levels was generally greater and more variable than at low-nutrient levels. Nitrous oxide concentrations did not differ markedly across the sampling campaigns, and seasonal trends showed no systematic pattern between campaigns (Fig. 1a,b).

Dissolved N_2O concentrations in the mesocosms in the high-nutrient treatment were generally greater than those in the low-nutrient treatment (Fig. 2a). The effect of the warming treatments on N_2O concentrations was not visible across and within nutrient treatments (Fig. 2b,c).

The scatter plots of dissolved N_2O concentrations vs. various environmental variables showed no clear visible relationship between the variables (Fig. 3; Supporting Information Fig. S1). Hence, there was no apparent link between N_2O concentration and water temperature, dissolved oxygen, total nitrogen, or nitrate (Fig. 3a–d).

We used linear mixed models to investigate the relationship between N_2O concentrations and the warming and nutrient treatments. The mixed model revealed a statistically significant positive association between N_2O concentrations and nutrient levels, whereas the effect of the warming treatments was not significant (Table 1, model 1). These results suggest that higher nutrient levels are associated with higher N_2O concentrations in the shallow lake mesocosms, while potential effects of warming were not identified. Another model (Table 1, model 2a) was used to assess potential linkages between N_2O concentration and selected environmental variables (nitrate, total iron, phosphate, water temperature, pH, oxygen, plant volume inhabited, and Chl *a*). Nitrous oxide concentrations were correlated positively with nitrate and total iron and negatively with oxygen. We also ran another model (Table 1, model 2b) including total nitrogen and total phosphorus instead of nitrate and phosphate. According to the latter model, N_2O concentrations were correlated positively with total nitrogen and total phosphorus, and negatively with oxygen and Chl *a*.

As a result of the nutrient treatment, the mean nitrate concentration was around 0.10 mg N L^{-1} at low-nutrient level, whereas it was 1.62 mg N L^{-1} at high-nutrient level (median 0.007 and 0.80 mg N L^{-1}, respectively) (Fig. 4a). For total nitrogen and total phosphorus, the mean concentration was 0.41 mg N L^{-1} and 0.019 mg P L^{-1} (median 0.35 mg N L^{-1} and 0.013 mg P L^{-1}) at low-nutrient level and 3.1 mg N L^{-1} and 0.22 mg P L^{-1} at high-nutrient level (median 2.82 mg N L^{-1} and 0.19 mg P L^{-1}). Despite the large differences in nutrient concentrations across nutrient treatments, the N_2O emission factors differed only slightly, with mean values of 0.028% and 0.038% at low- and high-nutrient levels (median 0.007% and 0.015%, respectively). According to the mixed effect model, the N_2O emission factors showed a weak significant relationship ($p = 0.032$, Table 1, model 3) with the nutrient treatments, greater values being associated with the

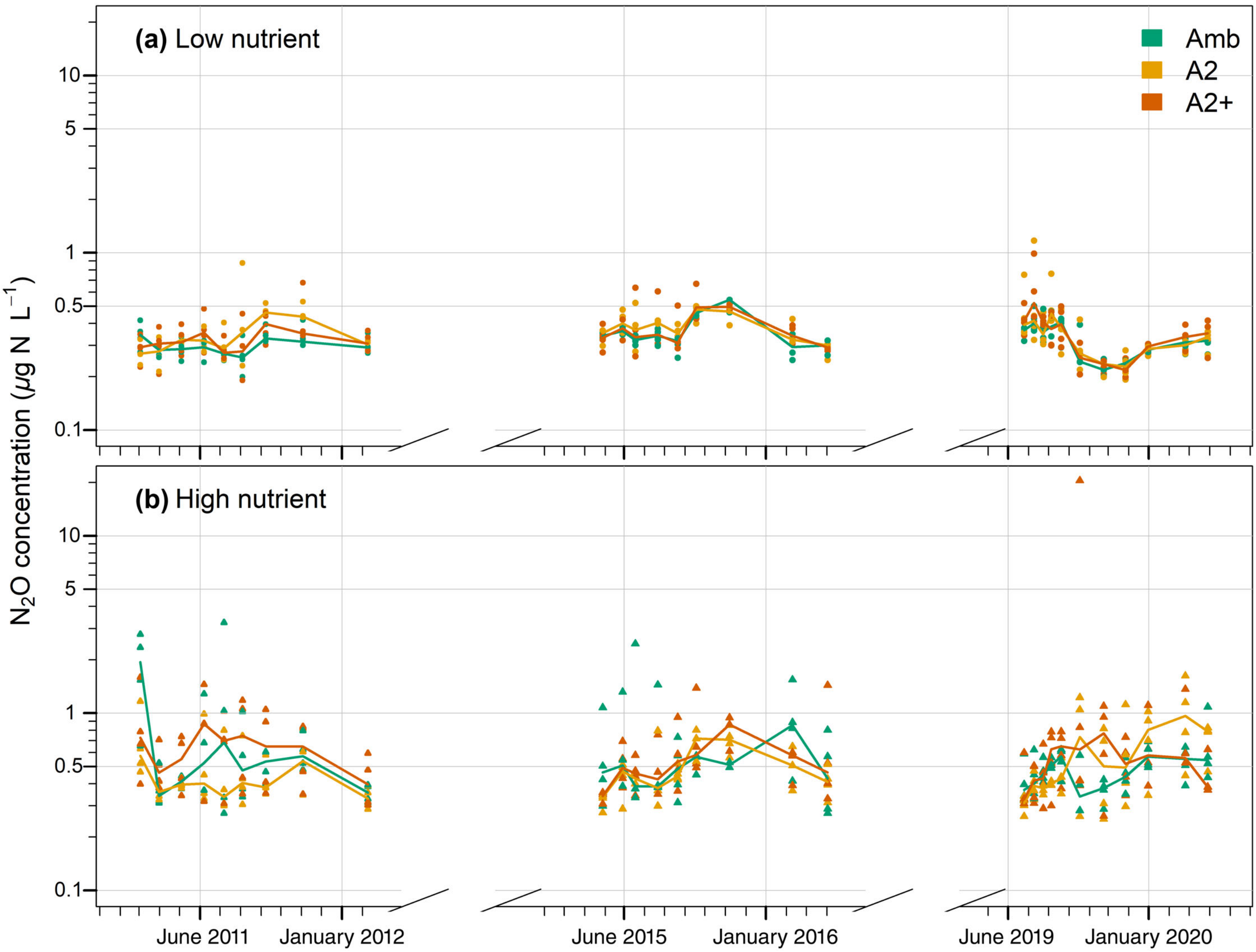

Fig. 1. Nitrous oxide (N_2O) concentration measured in the mesocosms over time and across the three different warming and nutrient treatments: (**a**) low nutrient and (**b**) high nutrient. Amb, unheated; A2, heated +2.5–4.0°C; and A2+, heated +4.75–6.0°C. Each point represents the N_2O concentration measured in one mesocosm, and the lines represent the mean N_2O concentration for each of the three warming treatments. All N_2O concentrations are temperature standardized (see methods). Note that the *y*-axis is log-transformed.

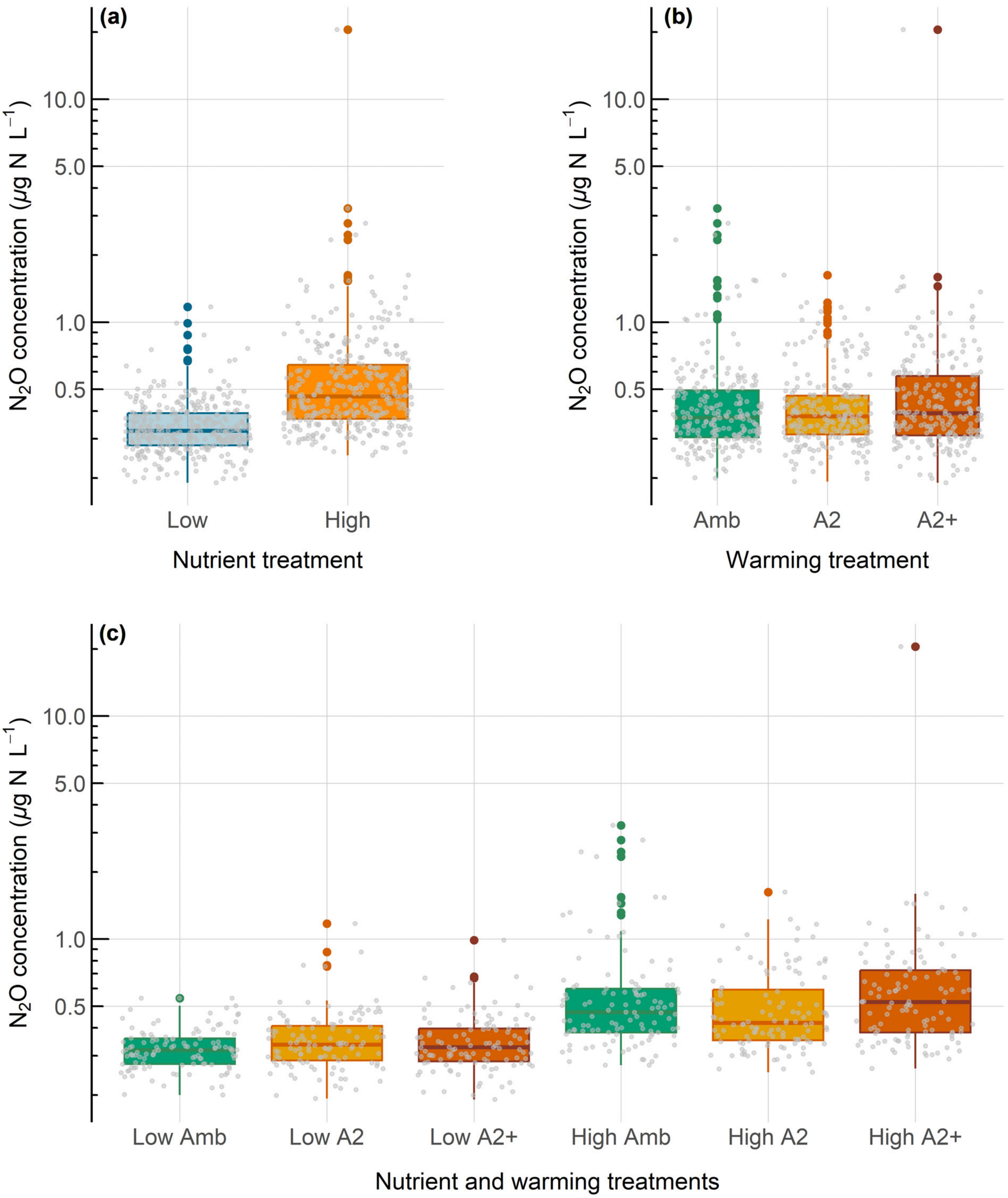

Fig. 2. Boxplots showing the nitrous oxide (N_2O) concentrations measured in the mesocosms across (**a**) nutrient treatments (low- or high-nutrient level), (**b**) warming treatments, and (**c**) combination of nutrient and warming treatments. Amb, unheated; A2, heated +2.5–4.0°C; and A2+, heated +4.75–6.0°C. The boxplots display interquartile range, median, and potential outliers. The central box represents the interquartile range, with the median as a horizontal line within it. Whiskers extend to the minimum and maximum values within 1.5 times the interquartile range. Outliers are depicted as individual points. Gray points represent the individual observations measured in the mesocosms. Note that the *y*-axis is log-transformed.

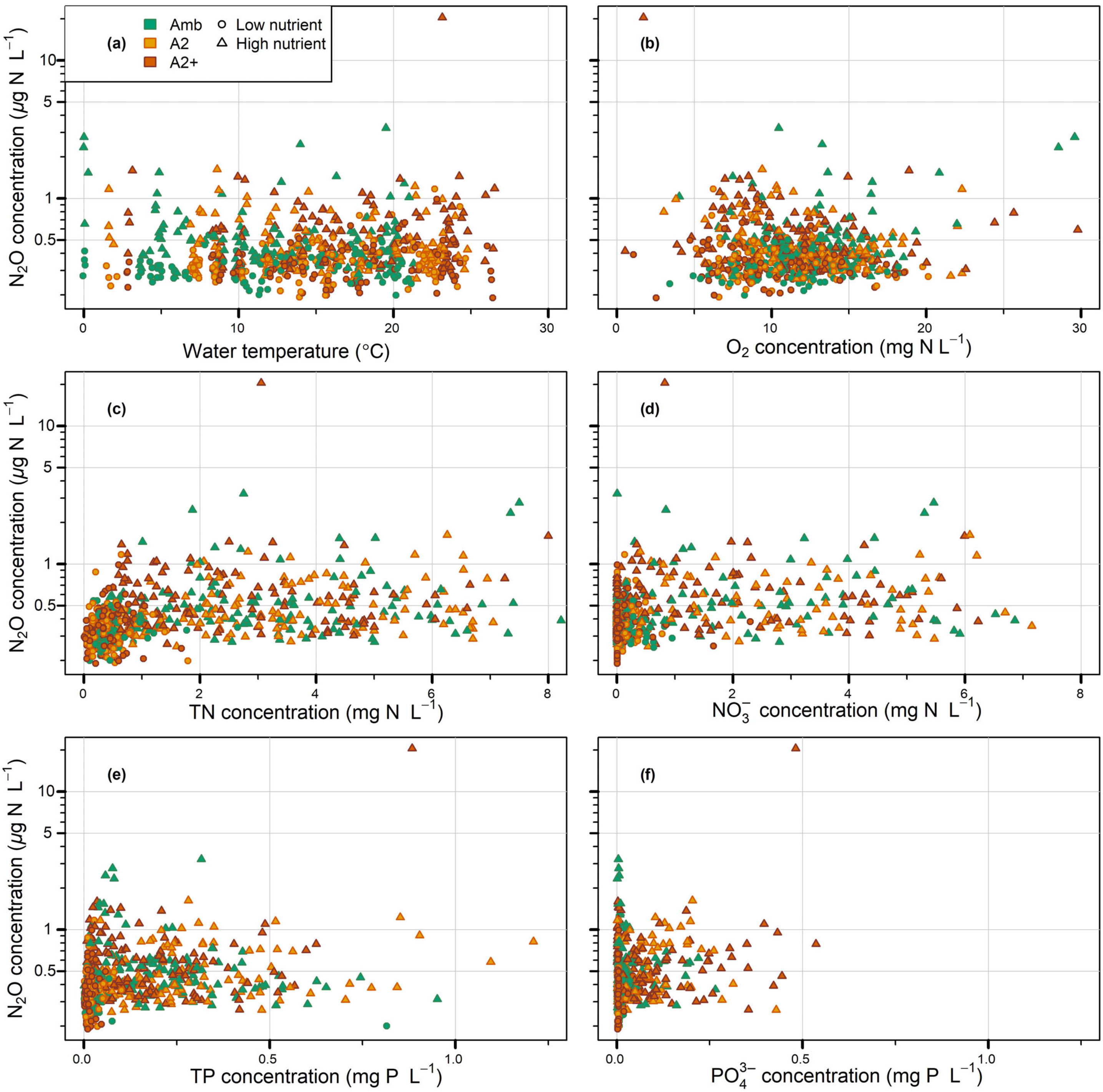

Fig. 3. Scatter plots showing nitrous oxide (N_2O) concentrations measured in the mesocosms relative to various environmental factors: (**a**) water temperature, (**b**) dissolved oxygen (O_2), (**c**) total nitrogen (TN) in the water, (**d**) nitrate (NO_3^-) in the water, (**e**) total phosphorus (TP) in the water, (**f**) phosphate (PO_4^{3-}) in the water. The different symbols and colors represent the different nutrient and warming treatments. Amb, unheated; A2, heated +2.5–4.0°C; A2+, heated +4.75–6.0°C. Note that the *y*-axis is log-transformed.

high-nutrient treatment, while the warming treatments had no significant effect (Fig. 4b). A mixed model was also used to identify the environmental variables significantly correlated with the N_2O emission factors (Table 1, model 4). We found that total nitrogen and total phosphorus had a positive effect on N_2O emission factors, whereas oxygen, water temperature, and total iron had a negative effect.

Table 1. Output of the linear mixed models used to explore the relationship between the response variable and N_2O concentration and the predictor variables in the warming treatment (unheated, heated A2, heated A2+) and the nutrient treatment (low, high). The fixed effects in models 2, 2b, and 4 were all log transformed.

Linear mixed models	Response variable	Fixed effects included in full model	Random effects	Parameter estimates	Value	SE	df	*t*-value	*p*-value	*N*
Model 1	Ln (N_2O)	Warming, nutrient	Mesocosm	(Intercept)	−1.110	0.053	662	−21.10	**<0.001**	686
				A2	−0.010	0.064	20	−0.16	0.8743	
				A2+	0.063	0.064	20	0.97	0.3429	
				High	0.429	0.053	20	8.17	**<0.001**	
Model 2	Ln (N_2O)	Nitrate, total iron, phosphate, oxygen, water temperature, pH, plant volume inhabited, chlorophyll *a* (Chl *a*)	Mesocosm	(Intercept)	−0.237	0.099	635	−2.39	**0.0171**	662
				Nitrate (mg N L^{-1})	0.051	0.006	635	8.55	**<0.001**	
				Total iron (mg L^{-1})	0.042	0.012	635	3.39	**<0.001**	
				Oxygen (mg L^{-1})	−0.176	0.037	635	−4.78	**<0.001**	
Model 2b	Ln (N_2O)	Total nitrogen, total iron, total phosphorus, oxygen, water temperature, pH, plant volume inhabited, Chl *a*	Mesocosm	(Intercept)	−0.593	0.099	634	−5.98	**<0.001**	662
				Total nitrogen (mg N L^{-1})	0.098	0.015	634	6.40	**<0.001**	
				Oxygen (mg L^{-1})	−0.114	0.037	634	−3.04	**0.0024**	
				Total phosphorus (mg P L^{-1})	0.037	0.018	634	2.08	**0.0377**	
				Chl *a* (mg L^{-1})	−0.028	0.012	634	−2.34	**0.0196**	
Model 3	Ln (EFN_2O + 0.0005)	Warming, nutrient	Mesocosm	(Intercept)	−7.369	0.051	662	−143.79	**<0.001**	686
				High	0.118	0.051	20	2.30	**0.032**	
				A2	0.026	0.063	20	0.41	0.684	
				A2+	0.070	0.063	20	1.12	0.276	
Model 4	Ln (EFN_2O + 0.0005)	Total nitrogen, total iron, total phosphorus, oxygen, water temperature, pH, plant volume inhabited, Chl *a*	Mesocosm	(Intercept)	−6.293	0.182	633	−34.51	**<0.001**	662
				Total nitrogen (mg N L^{-1})	0.086	0.023	633	3.78	**<0.001**	
				Oxygen (mg L^{-1})	−0.349	0.053	633	−6.58	**<0.001**	
				Total iron (mg L^{-1})	−0.115	0.022	633	−5.29	**<0.001**	
				Water temperature (°C)	−0.073	0.029	633	−2.49	**0.013**	
				Total phosphorus (mg P L^{-1})	0.060	0.023	633	2.63	**0.009**	

Note: Significant *p*-values are indicated in bold.

Discussion

Effect of nutrient enrichment and warming on N_2O concentrations

We used a unique experimental setup and a large dataset to investigate how nutrient enrichment and warming affect N_2O concentrations in lakes and ponds. Our results showed a significant positive effect of nutrient enrichment on N_2O concentrations and emissions, whereas warming had no discernable effect.

The effect of nutrient enrichment on N_2O concentrations is consistent with the literature (Palacin-Lizarbe et al. 2018; Zhou et al. 2021) and confirms the results from Davidson et al. (2015) who used the same experimental setup in 2011–2012. Notably, our expanded dataset encompassing three sampling campaigns over 9 yr provides a more comprehensive understanding of the factors controlling N_2O emissions. There is usually a positive correlation between nitrogen availability and N_2O concentrations or emissions, and this was also the case in our study despite considerable variability in the scatter plot, indicating that other factors were also at play. Indeed, besides nitrate or total nitrogen, we found a positive correlation with total iron and total phosphorus and a negative correlation with oxygen and Chl *a*. The correlation with total iron is probably due the more anoxic environment found in the high-nutrient mesocosms, which favors the release of iron from the anoxic sediment (Søndergaard et al. 2003). Total phosphorus contributes to a more eutrophic environment, but as both nitrogen and phosphorus were added simultaneously in the high-nutrient mesocosms, it is unclear whether phosphorus has a direct effect on N_2O emissions. While

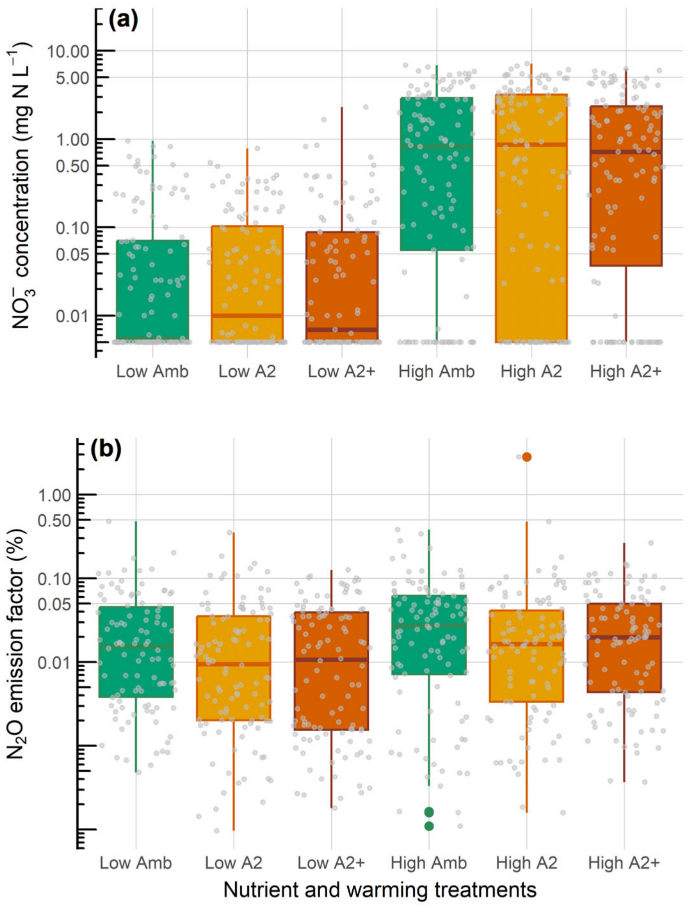

Fig. 4. (**a**) Nitrate concentrations in the different treatments and (**b**) nitrous oxide (N_2O) emission factor in the different treatments. The boxplots display interquartile range, median, and potential outliers. The central box represents the interquartile range, with the median as a horizontal line within it. Whiskers extend to the minimum and maximum values within 1.5 times the interquartile range. Outliers are depicted as individual points. Gray points represent the individual observations measured in the mesocosms. Amb, unheated; A2, heated +2–4°C; A2+, heated +4–6°C. Note that the *y*-axis is log-transformed.

the negative correlation with oxygen levels was anticipated due to the preference of denitrification for low-oxygen environments, the reason for the negative correlation with Chl *a* is less clear but might due to a depletion in nitrate availability when Chl *a* is higher.

Contrary to our hypothesis, neither the warming treatments nor the water temperature had a statistically significant impact on N_2O concentrations. Therefore, we recommend caution when transposing an effect derived from the temperature effect on individual biogeochemical processes to assert the effect of global warming. The lack of warming effect on N_2O emissions might be explained by a number of processes and controls affecting the nitrogen cycle; for example, at low substrate availability, a moderate increase in temperature may have only a limited effect on denitrification (Velthuis and Veraart 2022). We speculate that warming may also increase other processes such as the biological uptake by plants or the abundance of algae that may compete with denitrifiers or other nitrogen-processing microbes for substrate availability. For example, it is quite common for eutrophic lakes and ponds to have low-nitrate concentrations in summer when the temperature is the highest and nutrient loading is low (Kolzau et al. 2014; Søndergaard et al. 2017), possibly hindering N_2O production. Therefore, due to the competition between the different processes, an increase in temperature may not always result in increased N_2O emissions, as would be expected from the temperature effect on biogeochemical processes. Still, in a previous study, using the same mesocosm setup, it was observed that a summer heatwave increased the emission of N_2O, but that the long-term warming treatments had no significant effect (Audet et al. 2017). Hence, based on our dataset, we find no evidence of a synergy between nutrient enrichment and warming on N_2O emissions. While many lakes are warming, nitrogen availability appears to be the key factor in mitigating N_2O emissions, and the overall effect of warming seems limited (Palacin-Lizarbe et al. 2018).

Emission factors

Abundant reactive nitrogen availability poses a risk of increasing N_2O emission factors since the final step of denitrification, the conversion of N_2O to N_2, may be inhibited (Weier et al. 1993). Our study revealed a significant effect of nutrient treatment or total nitrogen on N_2O emission factors, although the difference was rather small (the emission factors were 0.028% and 0.038% at low- and high-nutrient levels) despite a substantial difference in nitrate concentrations (mean concentration in the high-nutrient treatment was 16 times higher than in the low-nutrient treatment). This result warrants cautious interpretation as it questions the prevailing notion that higher nutrient loads elevate emission factors. However, other environmental factors are probably involved as the models additionally revealed a potential influence of water temperature (negative effect), oxygen, total iron, and total phosphorus. These results underscore the intricate interplay between various factors influencing N_2O emission factors. Furthermore, our emission factors fall well below those for waterbodies defined by the IPCC (EF_{5r} 0.26%) (Hergoualc'h et al. 2019), supporting the idea that the IPCC values might be overestimated (Maavara et al. 2019). However, the uncertainty of our N_2O flux estimates remains high due to the limitation that the gas exchange constant *k* is derived from only one date, and the monthly sampling frequency may not have been frequent enough to capture the temporal variability in nitrous oxide concentrations.

Nitrous oxide sink

We found that the mesocosms sometime acted as N_2O sinks as evidenced by negative N_2O fluxes. Undersaturation occurs when N_2O consumption outpaces production, and there is a growing body of evidence that shallow lakes and ponds can act as N_2O sinks (Webb et al. 2019; Aho et al. 2023). A clear mechanistic understanding of the processes leading to the formation of N_2O sinks is still lacking, but proposed mechanisms include: (i) N_2O reduction as the last step of microbial denitrification and (ii) N_2O-dependent N fixation (Si et al. 2023). Undersaturation seems especially connected to periods with low-nitrogen concentrations, although N_2O uptake can also be observed when reactive nitrogen is available (Webb et al. 2019; Aho et al. 2023; Si et al. 2023). Our observations of N_2O sinks mostly occurred during the growing season in agreement with Webb et al. (2019) and Soued et al. (2016) for ponds and boreal lakes, respectively. However, Si et al. (2023) observed that the strongest N_2O undersaturation in experimental ponds occurred at colder temperature, while Aho et al. (2023) found no seasonality in lakes across the United States. These contrasting results highlight the need for an improved understanding of the mechanisms controlling N_2O consumption in aquatic ecosystems.

Mesocosm strengths and limitations and application to real lakes

A central question is how the processes described in this mesocosm-based study apply to real lakes and ponds. To understand and predict the impact of eutrophication and climate change on N_2O emissions from shallow lakes, it is crucial to investigate how the mechanisms governing N_2O emissions will respond to these disturbances within the intricate, interconnected framework of the ecosystem. Replicated mesocosm experiments offer a valuable experimental setup that strikes a balance between the necessary abstraction of reality and the ability to isolate experimental effects (Benton et al. 2007; Davidson et al. 2015). Our lake mesocosm climate change facility offers the opportunity to conduct our experiment in mesocosms having ecological assemblages well adapted to the different treatments as the mesocosms had been consistently heated and fertilized at the scenario levels for 8 yr prior to the 1^{st} N_2O sampling campaigns and for 16 yr for the last one. Hence, it was unlikely that we would get spurious transitory effects of warming or nutrient addition, which is a common pitfall of short-term laboratory and mesocosm experiments. However, our mesocosm setup with continuous mixing did not allow for stratification, a common feature for ponds and shallow lakes (Holgerson et al. 2022; Søndergaard et al. 2023; Davidson et al. 2024). Although our study examined nutrient concentrations and biological or physical parameters in the water column, it is likely that N_2O production occurs predominantly in the sediment. This limitation underscores the importance of studying both compartments for a comprehensive understanding.

Relevance and perspective

Our study suggests that reducing nutrient inputs is the primary driver to mitigate N_2O emissions from lakes and ponds and that warming may not necessarily lead to increased N_2O emissions, suggesting that focusing on reducing nitrogen losses is key to mitigating N_2O emissions from shallow fresh waters. Considering the steady global increase in the use of nitrogen fertilizer (Lu and Tian 2017), it is highly relevant to develop new policies and strategies to mitigate nitrogen losses and the subsequent N_2O emissions. Efforts targeting mitigation of N_2O emissions will most likely also be of benefit to the water quality and biodiversity of ponds and shallow lakes and improve their ecosystem resilience to warming.

Data availability statement

The data that support the findings of this study are available on Zenodo data repository 10.5281/zenodo.12698170.

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Conflict of Interest

None declared.

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