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Aquaculture farm largely increase indirect nitrous oxide emission factors of lake

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ABSTRACT

Freshwater aquaculture system is one of the main sources of nitrous oxide (N_2O) and must be accounted for when reporting indirect N_2O emissions from agricultural industries. The IPCC based approach recommend the indirect N_2O emission factors (EF_5) to estimate the emissions from freshwater. Unfortunately, the EF_5 for lake aquaculture, one of the most common freshwater aquacultures, has never been reported due to the scarcity of field data. To better understand the magnitude of EF_5 at lake aquaculture and identify the control factors, the EF_5 at aquaculture farm and open water (non-aquaculture region) of Lake Taihu were investigated based on long-term (2012–2017) in-situ field measurements. Our results showed the indirect N_2O emission at the aquaculture farm (1.52 ± 0.49 μmol m^{-2} d^{-1}) was over one order of magnitude higher than at the open water (0.12 ± 0.49 μmol m^{-2} d^{-1}). Furthermore, we also found large variability in the EF_5, which varied by one order of magnitude across time. The EF_5 was predicted by nitrogen and the mass ratio of carbon to nitrogen. The significantly higher mass ratio of DOC to DIN resulting from feed application contributed to substantial increase in the N_2O production efficiency and EF_5 at the aquaculture farm. The average EF_5 at the aquaculture farm and open water were 0.0021 ± 0.0013 and 0.0013 ± 0.0010, respectively. However, the measured EF_5 was lower than that IPCC's default value of 0.0025, implying IPCC method yielded the overestimated indirect emission, and large bias will occur when only use constant value considering the dramatic variability of observed EF_5.

1. Introduction

Increasing atmospheric nitrous oxide (N_2O) concentration has contributed to climate warming and stratospheric ozone depletion (Ravishankara et al., 2009; Tian et al., 2020). Agriculture is considered as the largest source of anthropogenic N_2O emission and contributed to the rapid growth of atmospheric N_2O concentration (Davidson, 2009; Tian et al., 2020). It is estimated that N_2O emissions from agriculture are 3.5–6.2 Tg N yr^{-1}, accounting for 60 % to approximately 80 % of gross anthropogenic emissions (Kroeze et al., 1999; Davidson and Kanter, 2014; Tian et al., 2020). Additionally, anthropogenic N_2O emissions from agriculture are divided into direct emissions and indirect emissions. The direct N_2O emissions from fertilized soils have been extensively documented with lots of measurements and models (Shcherbak et al., 2014; Shang et al., 2019; Cui et al., 2021). However, the indirect N_2O emissions from aquatic ecosystems due to reactive N leaching and runoff of agricultural fields are rarely studied (Turner et al., 2015; Audet et al., 2017; Yang et al., 2022).

The indirect N_2O emissions are essential to agricultural N_2O inventories due to substantial agricultural N inputs into aquatic ecosystems (Xiao et al., 2019a; Webb et al., 2021). Although relatively few measurements are conducted for indirect N_2O emissions of agricultural

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aquatic ecosystems (Audet et al., 2017; Hama-Aziz et al., 2017), it is estimated that the indirect emissions contribute to over 25 % of total agricultural N_2O emissions (Reay et al., 2012), indicating indirect emissions largely contributed to inter-annual variability of the total N_2O emission (Griffis et al., 2017). Thus, more field measurements of indirect N_2O emissions are required to improve the reliability of the emission from agriculture.

Freshwater aquaculture has become a primary global agricultural industry and is critical in ensuring food security. Unfortunately, as an essential component of agriculture indirect N_2O emission, the N_2O emissions from freshwater aquaculture systems are less understood due to the scarcity of field data (Hu et al., 2012; Yang et al., 2021). This knowledge gap in indirect N_2O emission of aquaculture has caused considerable uncertainty in the global N_2O budget estimates (Williams and Crutzen, 2010; Hu et al., 2012; Yuan et al., 2019). Aquaculture systems are maintained through daily supply of feeds including N loadings for improving production, but the majority of the external N are retained in the water and thereby can stimulate indirect N_2O emission (Hu et al., 2012; Chen et al., 2016; Yang et al., 2021). Global aquaculture production will increase significantly in the following decades with the increasing demand for protein due to the rapid population growth. Increasing aquaculture development will significantly release pollutants (Zhou et al., 2021), likely driving aquaculture systems to become primary anthropogenic sources of N_2O emissions (Xiao et al., 2019b). Considering the importance of N_2O emission, more field measurements in typical freshwater aquaculture systems are needed to provide accurate N_2O emissions from aquaculture.

IPCC (Intergovernmental Panel on Climate Change) provides emission factors (EF_5) to estimate indirect N_2O emissions from freshwaters (Hama-Aziz et al., 2017; Fu et al., 2018). The EF_5 was dimensionless and defined as the ratio of N_2O-N to the N loading input of water subject to leaching and runoff. This approach estimates the indirect N_2O emissions from freshwaters by multiplying a fraction of anthropogenic N loading with the default EF_5. The current EF_5 value for freshwaters such as rivers, groundwater, and estuaries are all set to 0.0025 (De Klein et al., 2006). However, nearly all studies demonstrate that the default EF_5 proposed by IPCC is highly uncertain because of large variability in environmental conditions (Maavara et al., 2019; Wu et al., 2021; Yang et al., 2021). Specifically, EF_5 not only varies by three orders of magnitude across different waterbodies (Webb et al., 2021), but also varies seasonally and spatially within a single waterbody (Qin et al., 2019; Xiao et al., 2019a). Thus, it is important and necessary to observe the temporal and spatial patterns of EF_5 for different waterbodies to accurately predict global indirect N_2O emissions. In fact, previous field measurements of EF_5 were mainly implemented in rivers/streams (Yu et al., 2013; Turner et al., 2015), reservoirs (Xiao et al., 2019a; Yang et al., 2022), and drainage ditches (Hama-Aziz et al., 2017). The measurements of EF_5 in freshwater aquaculture systems, especially for lake aquaculture, was less reported yet.

Lakes have been widely used for aquaculture in the past four decades in China due to the increasing demand for animal protein (Jia et al., 2013). It has become one of the most common agricultural industries in China with the peak area reaching 1 million ha as shown in a previous study, although the area of lake aquaculture decreased by 25 % in recent years (Pu et al., 2022). In fact, China's aquaculture sector ranks as the first and accounts for over 60 % of aquaculture production in the world (Pauly and Zeller, 2017). However, aquaculture lakes are likely to result in large N_2O emissions (Zhou et al., 2021), due to their semi-artificial system with large amount of feed rich in carbon and nitrogen for high fish yield purpose (Yang et al., 2021; Pu et al., 2022). The degradation of these organic-rich feed could increase N_2O production and stimulate indirect N_2O emissions (Yang et al., 2021; Zhou et al., 2021). It is proposed that lake aquaculture accounted for 89 % of the increase in N_2O emissions from Chinese aquaculture sectors (Zhou et al., 2021). However, our understanding of the magnitude of indirect N_2O emissions from lake aquaculture is limited due to the unknown EF_5 in this ecosystem.

In this study, the indirect N_2O emission factors EF_5 at an aquaculture farm (Dongtaihu Bay) and open water without aquaculture of Lake Taihu, the third largest freshwater lake in China, were investigated based on long-term (2012–2017) field measurements. Aquaculture activities had contributed to increases in nutrient loading at the aquaculture farm (Dongtaihu Bay; Qin et al., 2007) and may stimulate the indirect N_2O emissions. The main aims of our study are: (1) to quantify the magnitude of EF_5 values in the lake aquaculture, (2) to determine the spatial-temporal variability in EF_5 values and their relationships with environmental variables, and (3) to elucidate the impact of aquaculture on EF_5 using observations at the aquaculture farm and open water. To our best knowledge, our research appears to be the first study that reports the indirect N_2O emissions factors EF_5 at lake aquaculture, which would advance our understanding for indirect N_2O emission from agricultural industries.

2. Materials and methods

2.1. Study sites and field measurements

Lake Taihu, with a size of 2338 km^2 and a mean water depth of 1.9 m, is the third largest freshwater lake in China. It features a subtropical monsoon climate, with annual precipitation of 1122 mm and mean air temperature of 16.2 °C (Lee et al., 2014). The main study region of Dongtaihu Bay, which is located in southeast part of Lake Taihu, has a size of 130 km^2 and a mean water depth less than 1.5 m. The Dongtaihu Bay is characterized by good water quality and abundant submerged macrophytes (Xiao et al., 2017; Pu et al., 2022). Since 1984, the aquaculture farm in Dongtaihu Bay was constructed and then expanded rapidly, with a peak area of 107 km^2 (Qin et al., 2007; Pu et al., 2022). The main form of initial aquaculture is pen-fish-culture, and now the aquaculture has been replaced by a polyculture mode dominated by the Chinese mitten crab (*Eriocheir sinensis*), supplemented by black shrimp (*Macrobrachium nipponense*), and giant river prawn (*Macrobrachium rosenbergii*) due to the adjustment of aquaculture structure (Qin et al., 2007; Pu et al., 2022). The young carb fry (about 10 g ind^{-1}), black shrimp fry (about 0.5 g ind^{-1}), and giant river prawn (about 12 g ind^{-1}) were stocked at the density of 15, 000 ind ha^{-1}, 225, 000 ind ha^{-1}, and 12, 000 ind ha^{-1}, respectively, as reported in previous study (Pu et al., 2022). Additionally, pellet feed, fish meat, and corn seed were applied once per day from January to October with annual application rates of 640 kg C ha^{-1} yr^{-1}, 1580 kg C ha^{-1} yr^{-1}, and 4180 kg C ha^{-1} yr^{-1}, respectively.

The aquaculture farm of Dongtaihu Bay is a fine place to investigate the indirect N_2O emission factors EF_5 at lake aquaculture mostly due to the following two reasons. First, Dongtaihu Bay is the most mature waterbody for aquaculture in China; Second, intense aquaculture had changed the environment variables of Dongtaihu Bay such as providing more autochthonous organic substrate as shown in previous studies (Qin et al., 2007; Pu et al., 2022). Our field measurements were carried out on the aquaculture farm from 2012 to 2017. There were three sampling sites (Site A, Site B, and Site C; Fig. 1) at the aquaculture farm. We collected water samples seasonally at the three sits in spring (May), summer (August), autumn (November), and winter (February). The central zone with open water was non-aquaculture regions. For comparison, sample at three sampling sites (Site D, Site E, and Site F) of the open water (Fig. 1) were also carried out seasonally (February, May, August, and November) from 2012 to 2017. The open water without aquaculture can be treated as a reference region to discuss the potential effects of aquaculture activity on the EF_5. Additionally, indirect N_2O emissions from the open water of the lake occurred likely due to relatively high nutrient loading from different sources. For example, the nitrate nitrogen concentration of the open water was comparable to that aquaculture systems with high N input (Xiao et al., 2019; Yang et al., 2021).

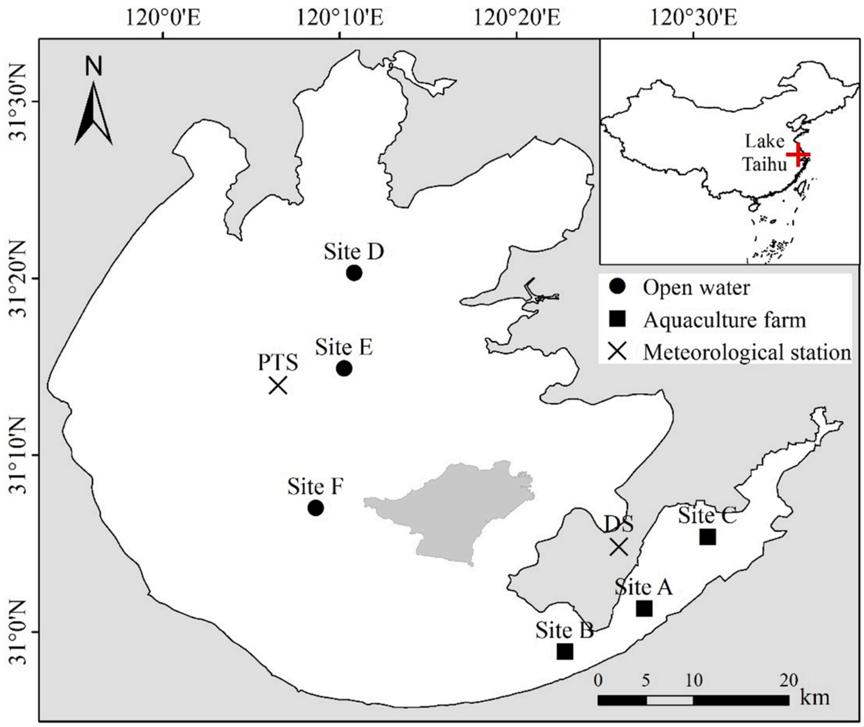

Fig. 1. Geographic location of Lake Taihu and the sampling sites at aquaculture farm and open water of the lake. Black square, black circle, and black cross indicate the sampling sites at the aquaculture farm (Site A, Site B, and Site C) and open water (Site D, Site E, and Site F), and meteorological station (PTS and DS), respectively.

2.2. Sample analysis and environmental variable acquisitions

Over the six-year period (2012–2017), water samples at the aquaculture farm and open water were collected for dissolved N_2O concentration analysis. The dissolved N_2O concentration was determined with head-space equilibration method (Davidson et al., 2015; Xiao et al., 2019b). Briefly, bubble-free surface water at the 20 cm depth was sampled in the field with a 300 mL glass bottle, and the glass bottle had been cleaned with local lake surface water prior to sampling. Then the bottle was sealed immediately and transported to laboratory for analysis. Ultrahigh purity N_2 gas (99.999 %) was injected into the glass bottle to create headspace for N_2O gas extraction to confirm the N_2O concentration. Then the glass bottle was shaken vigorously for five mins, which aims to allow the dissolved N_2O gas to reach equilibrium within the headspace. The N_2O gas sample in the headspace was then drawn and injected into a gas chromatograph. More details about the sampling and analysis have previously been reported (Xiao et al., 2017; Xiao et al., 2019a).

Environmental variables were included to confirm the magnitude, spatial-temporal variability, and main control factors of the EF_5. Surface water temperature (T_w), pH, dissolved oxygen (DO), total nitrogen (TN), total phosphorus (TP), dissolved inorganic nitrogen (DIN), dissolved organic carbon (DOC), and chlorophyll a (Chl-a) were considered in this study. The DIN included ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), and nitrite nitrogen (NO_2^--N). In the field, T_w, pH, and DO were measured with a multi-parameter probe, and the probe was calibrated prior to measurements. The probe for pH measurement had a precision of ± 0.01 pH units, and DO measurement had a precision of 0.1 mg L^{-1} (± 1 %) in the range of 0–20 mg L^{-1}. Water samples were collected for nutrient (TN, NH_4^+-N, NO_3^--N, NO_2^--N, and TP), DOC, and Chl-a analysis. The nutrient and DOC were measured with a spectrophotometer and TOC analyzer, respectively, and the Chl-a was determined spectrophotometrically. The sampling/measurement for these variables was conducted by TLLER (the Taihu Laboratory for Lake Ecosystem Research) and more details were reported in our previous studies (Xu et al., 2017; Zhang et al., 2018; Xiao et al., 2020b).

2.3. Flux calculations and indirect emission factors

The indirect N_2O emission (F_n) from surface water to the atmosphere was obtained with water-air gas exchange model (Cole and Caraco, 1998), which assumes that the dissolved N_2O at concentrations (C_w) above that of air saturation (C_{eq}) was subsequently emitted to the atmosphere. The calculation equation was:

$$F_n = k \times (C_w - C_{eq}) \quad (1)$$

where k is the gas transfer coefficient between the water-air interface. The wind speed measurements at 10 m height of PTS and DS (Fig. 1), two micrometeorological sites (Lee et al., 2014), were used for k calculation in this study. The detailed calculations of the gas transfer coefficient k were presented in our previous published papers (Xiao et al., 2017; Xiao et al., 2020b).

The indirect emission factors EF_5 from the lake aquaculture farm and open water in this study were calculated using the common IPCC (2006) methodology (De Klein et al., 2006), which has been broadly used for rivers (Turner et al., 2015; Hu et al., 2016; Qin et al., 2019), ditches (Hama-Aziz et al., 2017; Webb et al., 2021), lakes and reservoirs (Outram and Hiscock, 2012; Yang et al., 2022), and aquaculture ponds (Yang et al., 2021). The EF_5 was calculated as follows:

$$EF_5 = N_2O - N / NO_3^- - N \quad (2)$$

where the N_2O-N (mg L^{-1}) and NO_3^--N (mg L^{-1}) were concentrations in the surface water of aquaculture farm and open water.

2.4. Data processing and statistical analysis

The in-situ field data were separated into the aquaculture farm and open water (Fig. 1) to explore the potential effects of aquaculture on indirect N_2O emission factors. For temporal variability analysis, using all measurements within the corresponding zone from 2012 to 2017 to calculate a seasonal zonal mean. The total numbers of N_2O emission or EF_5 data for each zone was 24, where each data corresponded to one field survey throughout the 6-years sampling period. Additionally, field measurements in each sampling site were averaged over the 6-year survey period, and then the time-averaged quantities were used to perform spatial variability analysis.

Simple/multi-linear regression was conducted to determine the relationships between EF_5 and environmental variables. When multi-linear regression was carried out, the EF_5 and environmental variables were normalized in the range of 0–1, with 1 corresponding to the maximum value and 0 to the minimum value. Additionally, the variance inflation factor (VIF) in multi-linear regression was applied to determine if multi-collinearity was significant. If the value of VIF is greater than the threshold of 5, variables in the equation were removed to reduce multi-collinearity. A least significant difference post-hoc test was used to confirm the differences among measured/calculated variables, differences at the $p < 0.05$ level were determined as statistically significant.

3. Results

3.1. Environmental conditions

The water temperature was 18.6 ± 9.1 °C on the annual basis, showing minor spatial variability (Table 1). Specially, the water temperature differences among the six sampling sites were less than 0.8 °C annually. In contrast to water temperature, the bio-chemical variables varied largely (Table 1 and Fig. 2). The nutrients (e.g., TN, TP, and DIN) and Chl-a at the aquaculture farm were significantly ($p < 0.05$) lower than at open water, but significantly ($p < 0.05$) higher mass ratio DOC to DIN (DOC: DIN) occurred at the aquaculture farm (6.89 versus 9.52).

The two zones' bio-chemical variables varied seasonally (Fig. 2). The highest water temperature with mean value of 29.4 ± 2.8 °C occurred in summer and the lowest in winter (6.6 ± 2.0 °C). On the contrary, peak DO occurred in the winter and the lowest in the summer. Generally, the temporal variation of Chl-a was similar to water temperature, with a peak value in summer (aquaculture farm: 22.10 ± 10.94 μg L^{-1}; open water: 26.24 ± 8.15 μg L^{-1}) and trough in winter (aquaculture farm: 8.06 ± 2.05 μg L^{-1}; open water: 10.99 ± 3.50 μg L^{-1}). We need to note DIN did not show such seasonality with high DIN occurred in spring and winter.

3.2. Indirect N_2O emissions estimation

The indirect N_2O emissions across the water-air interface were calculated with Eq. (2). Based on the long-term field measurements, the indirect N_2O emission fluxes at the aquaculture farm and open water were 1.52 ± 0.49 μmol m^{-2} d^{-1} and 0.12 ± 0.13 μmol m^{-2} d^{-1}, respectively. Significantly ($p < 0.01$) higher indirect N_2O emissions occurred at the aquaculture farm. Additionally, the indirect N_2O emissions were highly positively correlated with the EF_5 across the six sampling sites (R^2 = 0.87, $p < 0.01$).

3.3. Spatial-temporal variations in EF_5

Our long-term (2012–2017) field measurements revealed that the EF_5 emission factors varied temporally (Fig. 3), which can vary by one order of magnitude across different sampling dates. Specially, the seasonal EF_5 ranged from 0.0005 to 0.0058 at the aquaculture farm, with a peak occurring in November 2014 and trough in May 2012, while the EF_5 ranged from 0.0002 in May 20130.0036 in November 2013 at open water. On average for the 6-years study period, the peak EF_5 occurred in autumn (aquaculture farm: 0.0033 ± 0.0015; open water: 0.0025 ± 0.0009), the trough occurred in spring (aquaculture farm: 0.0006 ± 0.0001; open water: 0.0025 ± 0.0002).

Substantial spatial variability in EF_5 between different zones was found over the 6-year field measurements (Fig. 4). The EF_5 at the aquaculture farm with an annual mean value of 0.0021 ± 0.0013 was significantly ($p < 0.05$) higher than that at open water (0.0013 ± 0.0010). Interestingly, the differences of EF_5 between sampling sites within each zone (the aquaculture farm and open water) were insignificant ($p > 0.05$; Fig. 4). Meanwhile, the temporal EF_5 of each sampling site within aquaculture farm (Site A versus Site B: R^2 = 0.56, $p < 0.01$; Site A versus Site C: R^2 = 0.50, $p < 0.01$; Site B versus Site C: R^2 = 0.44, $p < 0.01$) and open water (Site D versus Site E: R^2 = 0.31, $p < 0.01$; Site D versus Site F: R^2 = 0.46, $p < 0.01$; Site E versus Site F: R^2 = 0.52, $p < 0.01$) were highly correlated with each other.

3.4. Correlations between the EF_5 and environmental variables

The spatial-temporal variations in the indirect N_2O emission factors EF_5 could be predicted by some environmental variables. Temporally, it is worth noting that DOC and Chl-a were highly correlated with the EF_5 variability at the open water which indicates they can be used as predictors, but the patterns were not profound at the aquaculture farm (Figs. 5a and 6). In contrast to the DOC and Chl-a, the EF_5 were highly negatively correlated with DIN and the DOC:DIN at aquaculture farm and open water (Fig. 5). Spatially, the EF_5 was negatively correlated with DIN but positively correlated with the DOC:DIN (Fig. 7). Considering the significant role of DIN and DOC:DIN in the temporal variability in EF_5, nitrogen and carbon loadings regulate the EF_5 variability.

Although the variables used in Fig. 5 were correlated with each other, such as DIN was correlated with DOC:DIN at aquaculture farm (R^2 = 0.62, $p < 0.01$), multi-linear regression revealed that only normalized DOC:DIN and NH_4^+-N together explained 54 % of observed temporal variability in the normalized EF_5 at the aquaculture farm (R^2 = 0.54, $p < 0.01$). The multi-linear stepwise regression function was:

$$EF_5 = 0.77DOC : DIN + 0.45\mathrm{NH}_4^+ - N + 0.10 \quad (3)$$

However, normalized DOC:DIN and NH_4^+-N together explained 80 % of the variability of normalized EF_5 at the open water (R^2 = 0.80, $p < 0.01$):

$$EF_5 = 0.88DOC : DIN + 0.28\mathrm{NH}_4^+ - N - 1.40 \quad (4)$$

Table 1
Critical aquatic environment variables at aquaculture farm and open water of the lake during the field measurement period[a]. The data were presented as mean value ± one standard deviation.

Zone/Site		T_w	TN	TP	DIN	DOC	DOC:DIN	Chl-a
Aquaculture farm	Site A	18.6 ± 9.2	1.34 ± 0.54	0.06 ± 0.02	0.50 ± 0.24	3.50 ± 0.78	9.50 ± 6.58	11.77 ± 6.64
	Site B	18.5 ± 9.2	1.53 ± 0.64	0.06 ± 0.03	0.54 ± 0.35	3.39 ± 0.76	9.04 ± 5.60	10.65 ± 9.23
	Site C	18.6 ± 9.1	1.19 ± 0.31	0.05 ± 0.02	0.42 ± 0.17	3.84 ± 1.13	10.38 ± 4.23	15.24 ± 11.98
	All	18.6 ± 9.1	1.35 ± 0.42	0.06 ± 0.02	0.49 ± 0.24	3.54 ± 0.85	9.52 ± 4.97	12.55 ± 8.06
Open water	Site D	17.9 ± 8.9	2.13 ± 0.78	0.10 ± 0.03	0.80 ± 0.52	3.77 ± 1.03	6.92 ± 4.54	26.09 ± 27.81
	Site E	17.8 ± 9.0	2.07 ± 0.80	0.09 ± 0.03	0.79 ± 0.45	3.62 ± 0.78	6.79 ± 4.66	16.52 ± 10.33
	Site F	18.2 ± 9.2	1.96 ± 0.64	0.10 ± 0.03	0.68 ± 0.49	3.48 ± 0.77	7.43 ± 4.07	16.73 ± 12.95
	All	18.0 ± 9.0	2.05 ± 0.69	0.10 ± 0.03	0.75 ± 0.44	3.58 ± 0.84	6.89 ± 3.92	19.78 ± 12.95

[a] T_w, surface water temperature (°C); TN, total nitrogen concentration (mg L^{-1}); TP, total phosphorus concentration (mg L^{-1}); DOC, dissolved organic carbon (mg L^{-1}); DOC: DIN, mass ration of DOC (mg L^{-1}) to DIN (mg L^{-1}); Chl-a, chlorophyll a concentration (μg L^{-1}).

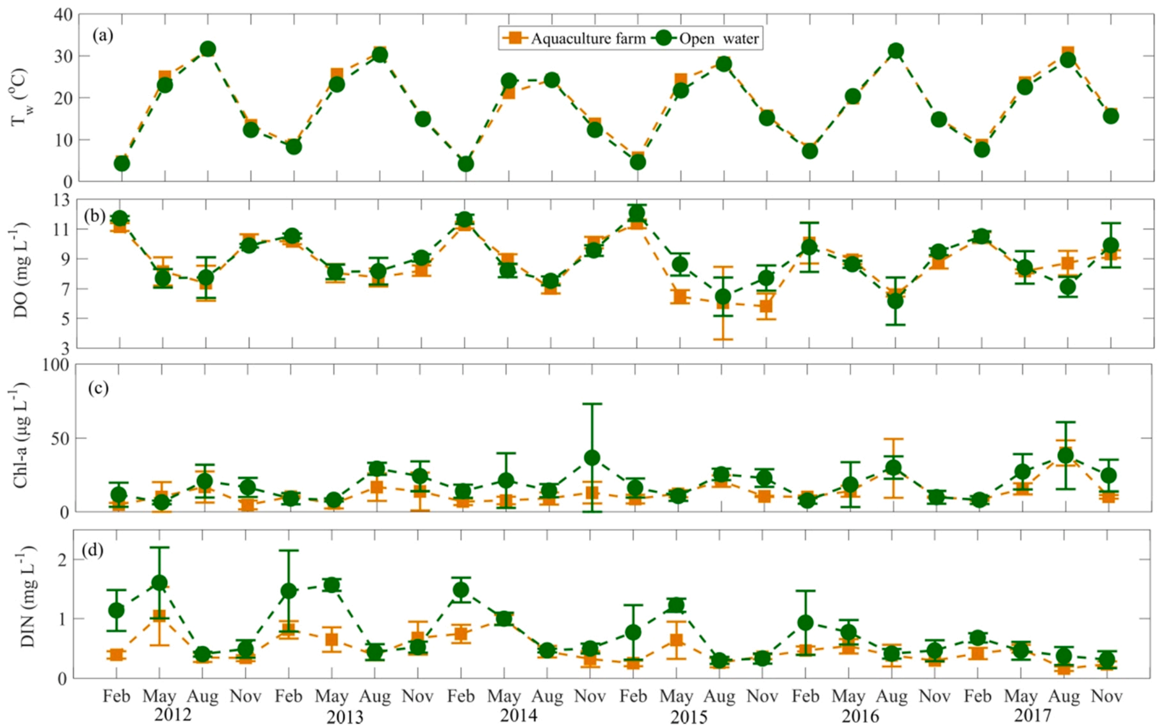

Fig. 2. Seasonal variations of water temperature (T_w, a), dissolved oxygen (DO, b), chlorophyll a (Chl-a, c), and dissolved inorganic nitrogen (DIN, d) at the aquaculture farm and open water of Lake Taihu from 2012 to 2017. Error bars indicate standard deviation.

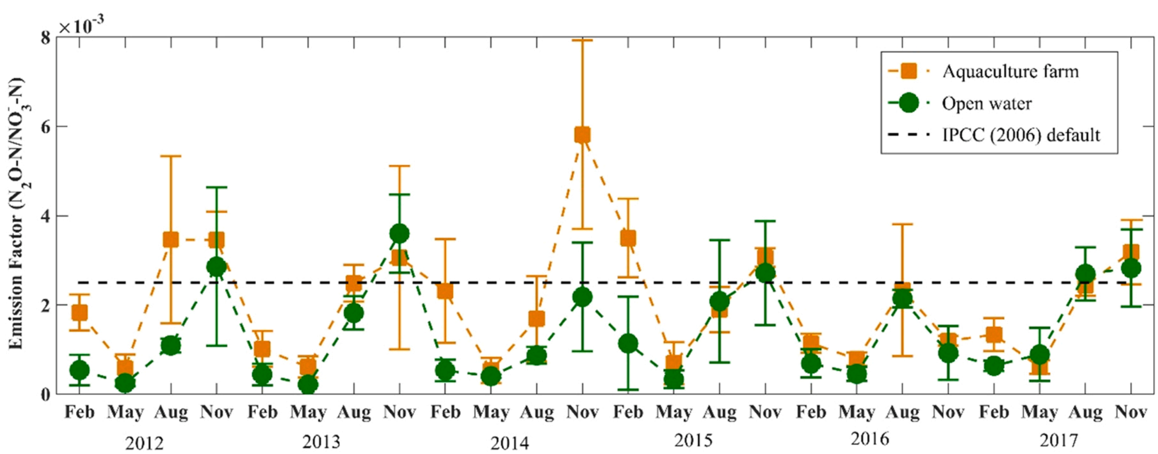

Fig. 3. Temporal variations in the indirect N_2O emission factors at the aquaculture farm and open water of Lake Taihu from 2012 to 2017. Error bars indicate standard deviation.

It was worth noting that the normalized DOC:DIN was unrelated with NH_4^+-N at both aquaculture farm and open water ($p > 0.05$), and the VIF between DOC:DIN and NH_4^+-N in Eq. (3) and Eq. (4) were 1.08 and 1.03, respectively, suggesting that multi-collinearity was negligible.

It is more informative to explore the factors influencing the dissolved N_2O concentration variability. Therefore, multi-linear stepwise regression was conducted to investigate relationships between dissolved N_2O concentration and environmental variables. Results showed that only DOC:DIN was the best predictor explaining the variability of the N_2O concentration at the aquaculture farm ($R^2 = 0.33$, $p < 0.01$), and only water temperature was the best predictor explaining the variability of the N_2O concentration at open water ($R^2 = 0.58$, $p < 0.01$).

4. Discussion

4.1. Factors influencing the EF_5 variability

The indirect N_2O emission factors EF_5 varied greatly across time and sites (Figs. 3 and 4). Consistent with previous studies in rivers and streams (Beaulieu et al., 2011; Hinshaw and Dahlgren, 2012; Xiao et al., 2019a), EF_5 in our study was negative with DIN (Figs. 5 and 7). Due to biological saturation, the inverse relationship between EF_5 and nitrogen loading may be attributed to decreased microbial activity with increased nitrogen loadings inputs (Mulholland et al., 2008; Xiao et al., 2019a; Yang et al., 2021). Additionally, the higher nitrogen loading in spring (Fig. 2) may result in the lowest EF_5 at that time (Fig. 3). Lower EF_5 occurred with higher nitrogen loadings implied less N_2O may be produced relative to DIN concentration, and we also supported the

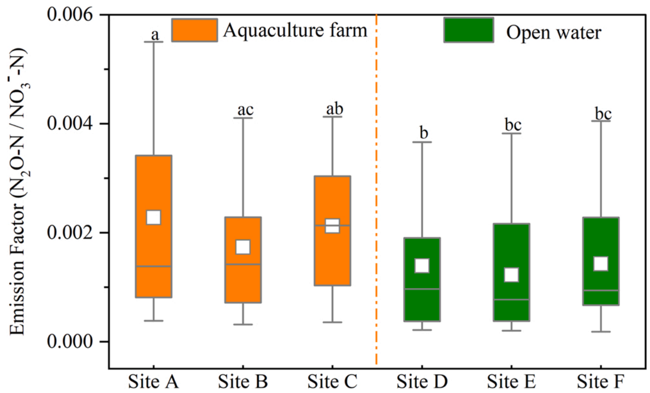

Fig. 4. Spatial variations of the indirect N_2O emission factors among different sites at the aquaculture farm and open water from 2012 to 2017. Different letters above the bars indicate significant ($p < 0.05$) differences between sampling sites.

assumption that N_2O production will non-linearly increase with rising DIN (e.g., NO_3^--N; Webb et al., 2021).

Carbon substrate was another critical factor influencing the EF_5 variability. Significant positive correlations between EF_5 and DOC or DOC:DIN were found (Figs. 5 and 7), suggesting N_2O production efficiency increased with augmented DOC concentration (Hu et al., 2016). Meanwhile, the dominated role of DOC:DIN in EF_5 also indicated that the available carbon relative to nitrogen was essential in regulating N_2O production efficiency. Generally, a high DOC:DIN ratio could improve the availability of labile carbon, increasing denitrification rates and N_2O production (Cooper et al., 2017; Capodici et al., 2018; Xiao et al., 2019b). A positive correlation between EF_5 and the mass ratio of carbon to nitrogen has been found in previous studies (Hu et al., 2016; Qin et al., 2019; Yang et al., 2021). Both of these implied the significant role of carbon substrate in regulating N_2O production processes and EF_5 variability.

As noted above, our 6-years observations revealed the seasonal variations of EF_5, which has not been examined before due to the lack of a dataset including seasonal measurements, especially those with long-term observations in previous studies (Qin et al., 2019; Webb et al., 2021). Apart from DOC and DIN, our seasonal field measurement from 2012 to 2017 found water temperature and Chl-a also regulated the EF_5 variability, which was less reported in previous studies (Hu et al., 2012; Outram and Hiscock, 2012). Besides, water temperature can affect the denitrification rate and stimulate N_2O production (Xiao et al., 2019b). The significant role of Chl-a in the EF_5 (Fig. 6) was a notable feature in our study. Considering algal blooms can increase labile DOC levels (Pacheco et al., 2014; Xiao et al., 2020a). The Chl-a, an indicator of algal abundance, can directly affect N_2O production and increase lake EF_5 by increasing carbon inputs.

4.2. *Effects of aquaculture on lake EF_5*

The aquaculture farm was a hot spot of atmospheric N_2O. Previous studies have demonstrated that lakes acted as N_2O sources, which should be considered in atmospheric N_2O budget estimation (McCrackin and Elser, 2011; Soued et al., 2016; Kortelainen et al., 2020). The estimated N_2O flux ranged from $-$ 5.24 μ mol m^{-2} d^{-1} to 14.99 μ mol m^{-2} d^{-1} across time and sites. The annual mean N_2O flux was 0.82 μ mol m^{-2} d^{-1}, showing the lake emitted N_2O to the atmosphere on average. Our results were also consistent with these previous studies above, which showed lakes were source of atmospheric N_2O. However, the indirect N_2O emission fluxes at the aquaculture farm (1.52 μmol m^{-2} d^{-1}) were over one order of magnitude higher than that at the open water (0.12 μmol m^{-2} d^{-1}) without aquaculture. Thus, aquaculture activities could significantly stimulate the lake N_2O production and emission, which should be paied more attention to better estimate indirect N_2O emissions from inland agricultural waters (Hu et al., 2012; Yuan et al., 2019). Additionally, the indirect N_2O emissions were highly positively correlated with the EF_5 ($R^2 = 0.87$, $p < 0.01$).

The annual mean EF_5 was 0.0021 at the aquaculture farm of the lake

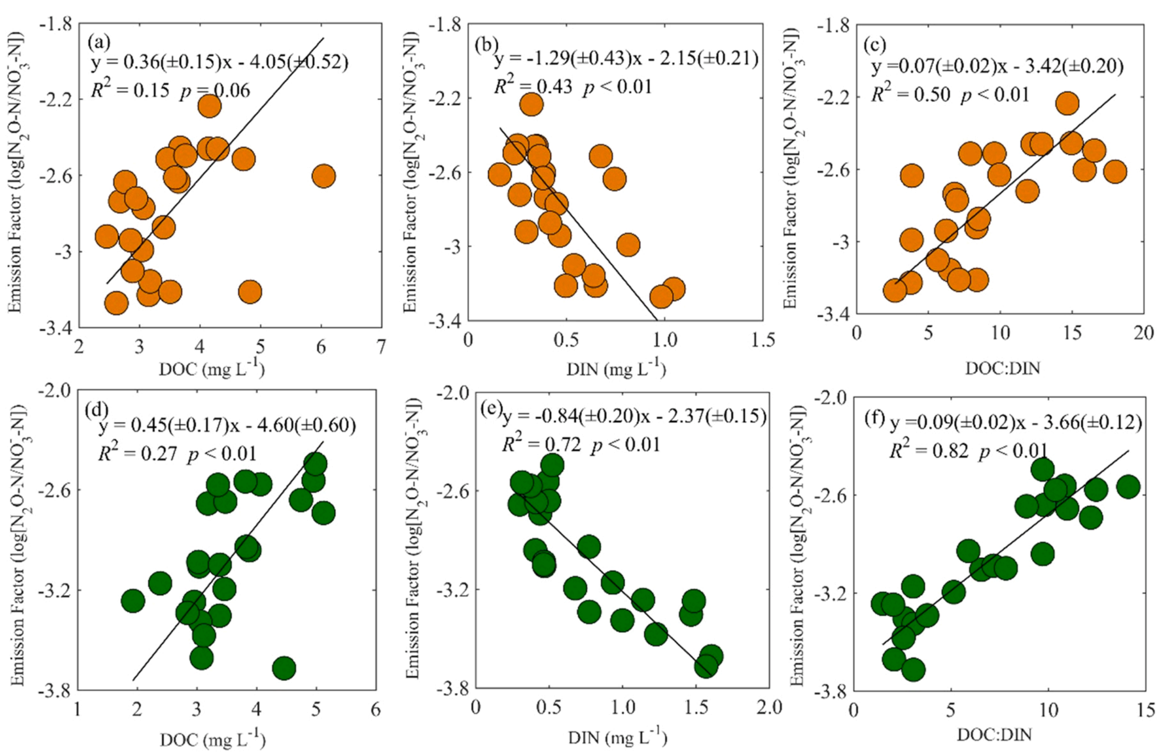

Fig. 5. Relationships between indirect N_2O emission factors and DOC, DIN, and the ratio of DOC (mg L^{-1}) to DIC (mg L^{-1}; DOC:DIN) at the aquaculture farm (a-c) and open water (d-f) based on the seasonal field measurements from 2012 to 2017. Parameter bounds on the regression coefficients indicate 95 % confidence limits.

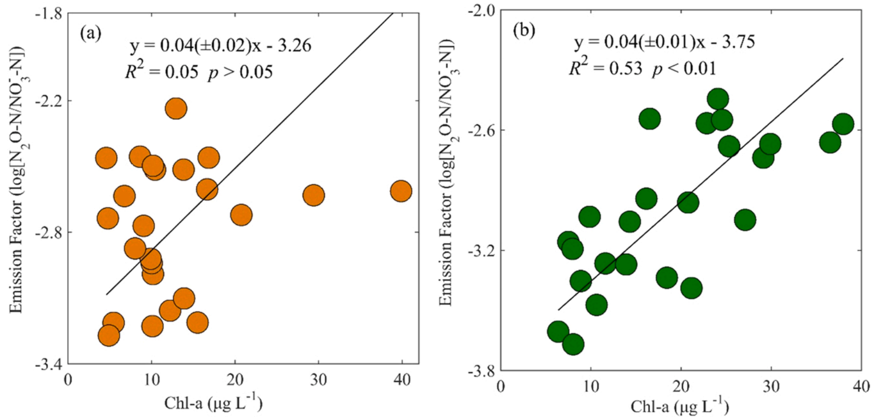

Fig. 6. Relationships between indirect N_2O emission factors and Chl-a at the aquaculture farm (a) and open water (b) of the lake based on the seasonal field measurements from 2012 to 2017. Parameter bounds on the regression coefficients indicate 95 % confidence limits.

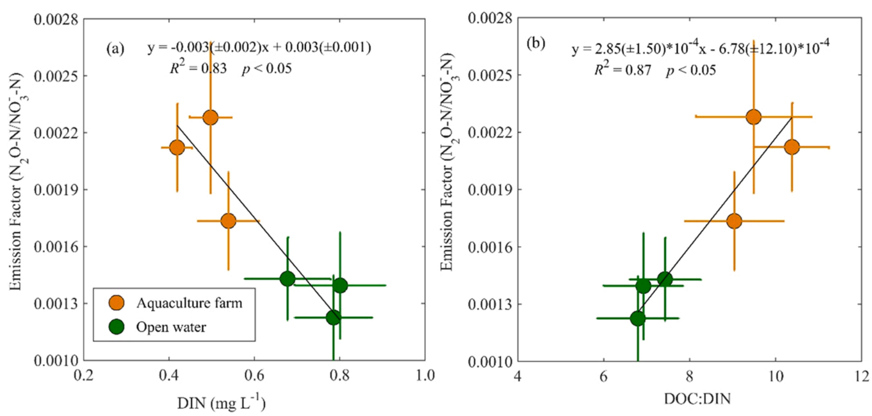

Fig. 7. Relationships between spatial mean indirect N_2O emission factors against DIN (a) and the ratio of DOC (mg L^{-1}) to DIC (mg L^{-1}; b) of the lake. Parameter bounds on the regression coefficients indicate 95 % confidence limits, and error bars indicate standard deviation. The yellow and green circles represented each spatial sampling site's annual mean value (2012–2017) at the aquaculture farm and open water, respectively.

based on the long-term field data from 2012 to 2017. The value was significantly ($p < 0.01$) higher than the annual mean values of 0.0013 at the open water without aquaculture (Fig. 3), 0.0007 reported for an open-water eutrophic zone (Xiao et al., 2019b), and 0.0008 for a lowland arable lake (Outram and Hiscock, 2012). These differences implied that lake aquaculture enormously affected the EF_5 of the water environment. Different EF_5 for different water types within the same watershed or the same water types between regions have been reported (Outram and Hiscock, 2012; Yang et al., 2021). This study found that the EF_5 varied considerably within a single lake due to the aquaculture activities.

The high mass ratio of carbon to nitrogen contributed to the large EF_5 at the aquaculture farm. The N_2O indirect emission factors EF_5 highly depended on the DOC:DIN (Figs. 5 and 7). The effect of DOC:DIN on the N_2O production has been explained above. The DOC:DIN at the aquaculture farm with an annual mean value of 9.52 ± 4.97 was significantly ($p < 0.01$) higher that at the open water (6.89 ± 3.92), which could stimulate the N_2O production substantially and increase the EF_5 (Outram and Hiscock, 2012; Hu et al., 2016). It is reported that about 6400 kg C ha^{-1} $year^{-1}$ as feed is added at the aquaculture farm (Pu et al., 2022), these feed with abundant starch and protein cannot be fully used by aquatic fish and is easy to decompose into carbon substrates (Yang et al., 2021), improving the labile carbon level relative to nitrogen. The study's findings implied that improving the efficiency of feed use efficiency could help to mitigate aquaculture N_2O production and emission.

4.3. *Implication of the measured EF_5*

The average EF_5 at the aquaculture farm of the lake (0.0021) was lower than the IPCC default value of 0.0025 by 16 %. This is consistent with the field measurement in many previous studies, as the agricultural river networks of China (Qin et al., 2019; Xiao et al., 2019a), rivers of the UK with different land use (Outram and Hiscock, 2012; Cooper et al., 2017), and ditches/drains in China and Sweden (Audet et al., 2017; Tian et al., 2018). The above studies also concluded the IPCC default value of 0.0025 may overestimate the indirect N_2O emission from water environment. Meanwhile, a recent study with mechanistic modeling also found IPCC default EF_5 likely overestimates the indirect N_2O emission (Maavara et al., 2019). Thus, the magnitude of EF_5 for different waterbody should be quantified to accurately estimate the indirect N_2O budget from aquatic ecosystem.

However, many studies have shown the IPCC default value is underestimated. The EF_5 value in maricultural ponds (Yang et al., 2021), the lakes receiving atmospheric nitrogen deposition (McCrackin and Elser, 2011), hydroelectric reservoirs in the northern boreal zone (Huttunen et al., 2002), and streams/rivers in the US Corn Belt (Turner et al., 2015) is about one order of magnitude higher than IPCC default value. These significantly higher EF_5, together with those significantly lower values, further demonstrated that the EF_5 varied greatly across regions (Hu et al., 2016; Webb et al., 2021). It is worth noting that the EF_5 could be predicted by the ratio of carbon to nitrogen in our study (Figs. 5 and 7), which likely provided a potential methodology to optimize the magnitude of EF_5 in specific waterbody.

Some efforts have already revised the IPCC default EF_5 to estimate the indirect N_2O emission accurately (Yu et al., 2013; Turner et al., 2015; Maavara et al., 2019). Unfortunately, most of the published studies are of limited usefulness for the revision of EF_5 due to relatively short-term investigations, as summarized by Qin et al. (2019). Substantial inter-annual and monthly/seasonal variability in the EF_5 has been found in our study (Fig. 4) and others (Qin et al., 2019; Wu et al., 2021; Yang et al., 2021). Clearly, the revision of EF_5 magnitude requires long-term observations with a high temporal resolution to develop accurate national indirect N_2O emission inventories, especially for the agriculture-impacted inland water.

4.4. Study limitations and future outlook

We should note there are several limitations in the presented research. Our study was consistent with previous studies showing aquaculture was a hot spot for atmospheric N_2O emission (Hu et al., 2012; Yuan et al., 2019). A recent study found an enormous contribution of the non-aquaculture period to the total N_2O emissions from aquaculture (Yang et al., 2020). However, the EF_5 differences between different periods (aquaculture versus non-aquaculture) at the aquaculture farm were hard to study due to the lacking information of detailed polyculture aquaculture practice mode as suggested above. Meanwhile, the EF_5 differences between the aquaculture farm and the open water varied greatly across seasons (Fig. 3), which were associated with water temperature ($R^2 = 0.17$, $p < 0.05$) and DO ($R^2 = 0.25$, $p < 0.05$). However, the potential mechanistic processes were less studied due to the lack of nitration and denitrification rates measurements. Further work should be carried out to help reduce aquaculture N_2O emissions for developing climate-resilient sustainable aquaculture.

5. Conclusions

To better understand the magnitude of indirect N_2O emission factors EF_5 at lake aquaculture and identify its control factors, the EF_5 at the aquaculture farm and open water without aquaculture of Lake Taihu were investigated based on long-term (2012–2017) field measurements. Our results showed the indirect N_2O emission at the aquaculture farm (1.52 μmol m^{-2} d^{-1}) was over one order of magnitude higher than at the open water (0.12 μmol m^{-2} d^{-1}) without aquaculture. Thus, aquaculture activities could significantly stimulate lake N_2O production and emission.

Our field measurements showed the average EF_5 was close but slightly lower than the IPCC default value of 0.0025. Long-term data showed the measured EF_5 varied greatly across zones and seasons. The large annual and seasonal variations of observed EF_5 indicate considerably bias can be leaded when only use constant default EF_5 to estimate indirect N_2O emissions from aquaculture waters. Spatially, the EF_5 with an annual mean value of 0.0021 was significantly ($p < 0.01$) higher than at the lake open water without aquaculture (0.0013). Seasonally, the EF_5 can vary by one order of magnitude across different sampling dates. Specially, the seasonal EF_5 ranged from 0.0005 to 0.0058 at the aquaculture farm and ranged from 0.0002 to 0.0036 at open water.

The EF_5 was negatively correlated with DIN but positively correlated with DOC: DIN, implying available carbon relative to nitrogen played a crucial role in regulating the N_2O production efficiency. Significantly higher DOC:DIN mass ratio due to feed conversion rate at the aquaculture farm contributed to the large EF_5. Our study appears to be the first one to investigate the EF_5 at lake aquaculture, which would advance our understanding of indirect N_2O emissions from agricultural industries.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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