



Hypoxia-Enhanced N₂O Production Under Ocean Acidification in the Bohai Sea

Ting Gu^{1,2†}, Dai Jia^{1,2†}, Xing Ma³, Liying Peng^{1,2}, Guicheng Zhang^{1,2}, Yuqiu Wei⁴, Tingting Lou^{3*} and Jun Sun^{1,5*}

¹ Research Centre for Indian Ocean Ecosystem, Tianjin University of Science and Technology, Tianjin, China, ² Tianjin Key Laboratory of Marine Resources and Chemistry, Tianjin University of Science and Technology, Tianjin, China, ³ Animal, Plant and Foodstuffs Inspection Center, Tianjin Customs, Tianjin, China, ⁴ Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, China, ⁵ College of Marine Science and Technology, China University of Geosciences, Wuhan, China

OPEN ACCESS

Edited by:

Yunping Xu,
Shanghai Ocean University, China

Reviewed by:

Kunpeng Zang,
Zhejiang University of
Technology, China
Xiao Ma,
GEOMAR Helmholtz Center for Ocean
Research Kiel, Germany

*Correspondence:

Jun Sun
phytoplankton@163.com
Tingting Lou
loutingtingucas@126.com

[†]These authors have contributed
equally to this work

Specialty section:

This article was submitted to
Marine Biogeochemistry,
a section of the journal
Frontiers in Marine Science

Received: 14 April 2021

Accepted: 22 June 2021

Published: 23 July 2021

Citation:

Gu T, Jia D, Ma X, Peng L, Zhang G,
Wei Y, Lou T and Sun J (2021)
Hypoxia-Enhanced N₂O Production
Under Ocean Acidification in the Bohai
Sea. *Front. Mar. Sci.* 8:695105.
doi: 10.3389/fmars.2021.695105

Nitrous oxide (N₂O) is a powerful greenhouse gas that degrades ozone. Hypoxia and ocean acidification are becoming more intense as a result of climate change. The former stimulates N₂O emissions, whereas the effects of the latter on N₂O production vary by the ocean. Hypoxia and ocean acidification may play a critical role in the evolution of future oceanic N₂O production. However, the interactive effects of hypoxia and ocean acidification on N₂O production remain unclear. We conducted a research cruise in the Bohai Sea of China to assess the occurrence of ocean acidification in the seasonal oxygen minimum zone of the sea and further conducted laboratory incubation experiments to determine the effects of ocean acidification and hypoxia on N₂O production. When pH decreased by 0.25, N₂O production decreased by 50.77 and 72.38%, respectively. In contrast, hypoxia had a positive impact; when dissolved oxygen (DO) decreased to 3.7 and 2.4 mg L⁻¹, N₂O production increased by 49.72 and 278.68%, respectively. The incubation experiments demonstrated that the coupling of ocean acidification and hypoxia significantly increased N₂O production, but, individually, there was an antagonistic relationship between the two. Structural equation modeling showed that the total effects of hypoxia treatment on N₂O production changes weakened the effects of ocean acidification, with overall positive effects. Generally speaking, our results suggest that N₂O production from the coastal waters of the Bohai Sea may increase under future climate change scenarios due to increasingly serious ocean acidification and hypoxia working in combination.

Keywords: ocean acidification, hypoxia, N₂O emission, Bohai Sea, oxygen minimum zone

INTRODUCTION

Nitrous oxide (N₂O) is an important greenhouse gas with a warming effect 265 times that of carbon dioxide (CO₂) (Stocker et al., 2013) and with the ability to destroy atmospheric ozone through photochemical reactions, resulting in significant impacts on global climate change (Crutzen and Ehhalt, 1977; Freing et al., 2012). N₂O emissions from oceans are the second-largest natural source of atmospheric N₂O and account for ~30% of total natural emissions (Bange, 2006), particularly N₂O emissions from the upper ocean (Nevison et al., 1995), making oceans important contributors

to global climate regulation (Qin, 2014). However, N₂O emissions are not uniformly distributed in the upper ocean due to complicated marine environments. Moreover, the effects of global climate change, including ocean acidification, temperature rise, and oxygen depletion, are likely to disrupt the balance (Codispoti, 2010).

Marine ecosystems play an important role in regulating global climate change through their strong regulatory capacity (Naqvi et al., 1998). Offshore low-oxygen areas are often caused by an increase in the flux of land-based nutrients into the sea and the eutrophication of estuaries and inshore waters. Seasonal stratification of seawater further hinders the transfer of oxygen from the upper layer of seawater to the bottom, producing seasonal variability in offshore hypoxic zones (Zhai et al., 2012; Zhang et al., 2016). Since hypoxic environments can stimulate the production and release of N₂O, hypoxic zones are hot spots for global marine N₂O emissions, affecting local and global climate. These areas have become an important subject of research into N₂O emission fluxes and mechanisms in minimum-oxygen zones within open oceans (Naqvi et al., 2000; Kalvelage et al., 2013; Arévalo-Martínez et al., 2015; Babbín et al., 2015; Ji et al., 2015; Kock et al., 2016; Trimmer et al., 2016).

The increase in global temperature due to the enhanced greenhouse effect has been balanced by the exchange of ~25% of anthropogenic CO₂ into the oceans (Le Quéré et al., 2014). Ocean acidification is the result of the ocean hosting excess CO₂, leading to changes in the carbonate system of upper ocean water (Orr et al., 2005). Over the past 20 years, this has resulted in a decrease in seawater pH of ~0.0011–0.0024 units per year, with the average marine pH being ~0.1 unit lower than before the Industrial Revolution (Stocker et al., 2013). Current research on biogeochemical cycling processes has shown that increasing pCO₂ and subsequent pH decline in the oceans are expected to affect microbial nutrient cycling directly and indirectly, for example, by increasing *Trichodesmium*-fixed N₂ and CO₂ (Hutchins et al., 2007) and changing ammonia-oxidizing archaea (AOA) abundance that indirectly alters N₂O emissions (Rees et al., 2016). Beman et al. (2011) studied changes in the nitrification rate under ocean acidification, showing that the nitrification rate decreased significantly while the pH dropped to the predicted value ($\Delta\text{pH} = 0.2$) of the future ocean. The N₂O produced by nitrification could be reduced by 0.06–0.83 Tg N year⁻¹ in the next 20–30 years under future ocean acidification. However, predictions of changing N₂O production in ocean acidification scenarios should also consider the effects of ocean acidification on denitrification. Although increasing CO₂ could have a negative effect on denitrification (Wan et al., 2016), it remains unclear how ocean acidification affects N₂O production.

Most known coastal anoxic areas appear in semi-enclosed areas prone to water stratification (Naqvi et al., 2010). The Bohai Sea of China, a shallow semi-enclosed inland sea, is typical of such eutrophic water bodies (Li et al., 2015). Rapid industrial and agricultural development in the region has resulted in high nutrient input into the Bohai Sea, such that the nitrogen and phosphate content exceeds Chinese standards and the eutrophic area has continuously increased (Liu and Yin, 2010). Zhai et al. (2012) and Zhang et al. (2016) found low DO and pH over a wide

range of the bottom of Bohai Sea, including a total area with DO < 3.0 mg L⁻¹ of ~4.2 × 10³ km². These spatial characteristics are consistent with a double-center cold-water structure (Lin et al., 2006; Zhou et al., 2009).

In this study, we designed a laboratory incubation experiment, investigating the effects of ocean acidification and hypoxia on seawater N₂O production in Bohai Bay, and then conducted aggregated boosted tree (ABT) and structural equation model (SEM) analyses to examine potential factors affecting N₂O production. This study was designed to verify two hypotheses: (1) Ocean acidification alone (Acid) reduces seawater N₂O production, while both hypoxia alone (Hyp) and ocean acidification with hypoxia (Acid + Hyp) conditions increase seawater N₂O production, and (2) hypoxia and ocean acidification change N₂O production by altering seawater properties rather than directly affecting N₂O production. A better understanding of changes in N₂O production and its mechanisms in the Bohai Sea hypoxic zone under future ocean acidification can provide data for improved prediction models.

MATERIALS AND METHODS

Sample Collection and Experimental Manipulation

A marine survey was conducted in August 2017 (**Figure 1**). Seawater depth at sampling stations ranged from 6 to 27 m, with the deepest at station A5 (**Figure 1**). Seawater samples for N₂O, pH, and DO were collected, using 5-L Niskin bottles, and vertical profiles of DO and pH were measured simultaneously with the Maestro multiparameter sensor (RBR Maestro 3). Temperature and salinity sensors were calibrated prior to the survey, and DO and pH sensor data were corrected by measured data before use. Samples for the incubation experiment were calibrated on December 21, 2017, at 117°48'11"E, 38°58'41"N (station A: relatively low high-quality exogenous substance input) and 117°43'23"E, 39°55'3"N (station B: relatively high quality of exogenous substance input because of human activity, especially nitrogen). The 5-L polycarbonate culture bottles filled with seawater were airtight and were kept in the dark for 2 h when transporting back to the laboratory. Experiments were performed with the experimental platform already set up and four target pCO₂ and oxygen levels, with triplicate samples per treatment (**Figure 1**, **Table 1**). All treatments were manipulated by gentle bubbling *via* plastic diffusers at uniform rates with commercially prepared air (CO₂:O₂ mixture) using acid-washed tubing. The bottle caps were customized for gas inlet/outlet and connected to the air mixture. The experiments were equilibrated for 6 h, distributed in 5-L polycarbonate bottles, sealed for 64 h, and maintained in a laboratory incubator at *in situ* temperature (15°C) in the dark (**Table 1**). Seawater CO₂ parameters were verified by measuring pH and dissolved inorganic carbon (DIC).

N₂O Analysis

Triplicate samples were collected using acid-cleaned Tygon tubing by siphoning from 5-L incubation bottles into

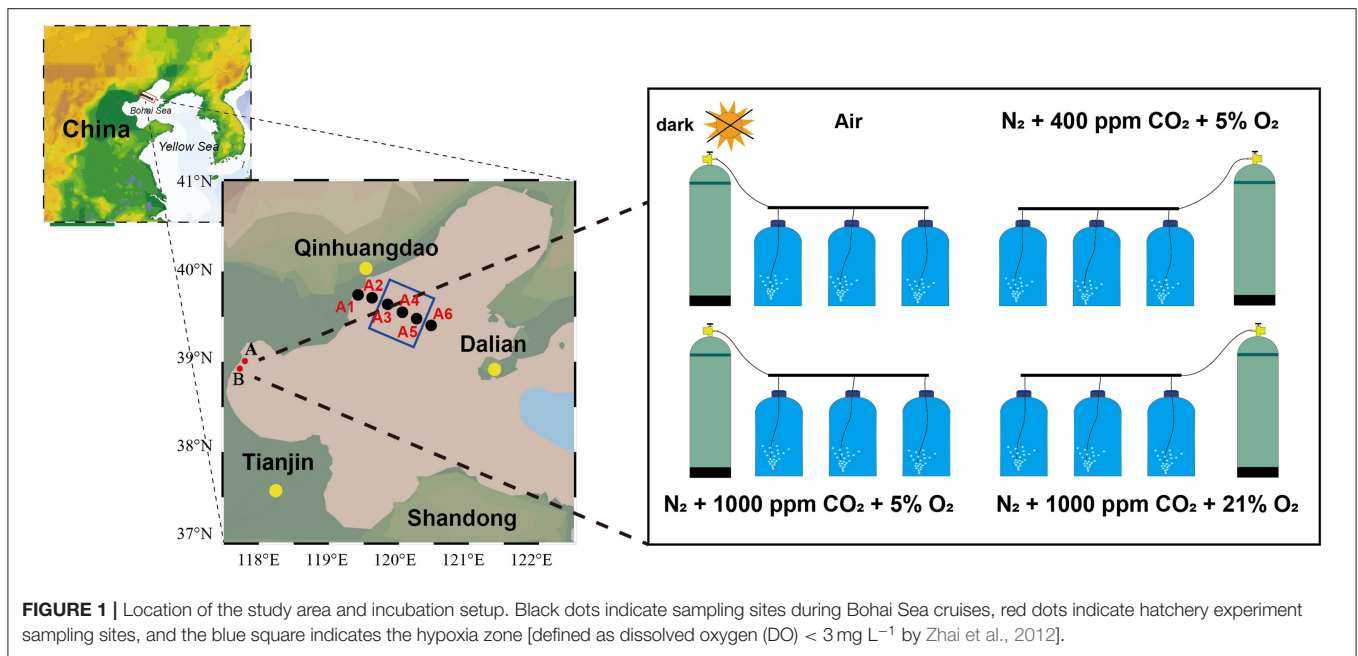


TABLE 1 | Overview of experiments, including sampling locations, treatments (Con, control; Hyp, hypoxia; Acid, acidification; Hyp+Acid, combination of hypoxia and acidification), and components of bubbling gas, pH, and dissolved oxygen (DO).

Location	DIN ($\mu\text{mol L}^{-1}$)	Treatment	Component of bubbling gas	pH	DO (mg L^{-1})
A	15.09 \pm 2.85	Con	Air	7.91 \pm 0.10	8.7 \pm 0.2
		Hyp	N ₂ +5%O ₂ +400ppmCO ₂	7.94 \pm 0.01	3.7 \pm 0.5
		Acid	N ₂ +21%O ₂ +1000ppmCO ₂	7.69 \pm 0.03	8.9 \pm 0.1
		Hyp+Acid	N ₂ +5%O ₂ +1000ppmCO ₂	7.76 \pm 0.05	3.7 \pm 0.1
B	63.91 \pm 3.69	Con	Air	7.90 \pm 0.01	6.7 \pm 0.3
		Hyp	N ₂ +5%O ₂ +400ppmCO ₂	7.95 \pm 0.04	2.4 \pm 0.2
		Acid	N ₂ +21%O ₂ +1000ppmCO ₂	7.71 \pm 0.02	6.9 \pm 0.2
		Hyp+Acid	N ₂ +5%O ₂ +1000ppmCO ₂	7.69 \pm 0.07	2.2 \pm 0.4

acid-washed 60-ml glass vials. Samples were allowed to overflow for two times the volume of the bottle to eliminate air bubbles. Samples were treated with 0.1 ml of saturated mercuric chloride and sealed with butylene rubber stoppers and an aluminum crimp seal. The rubber plug of the sample bottle was first penetrated by an injection syringe; then, 5.0-ml-high purity N₂ (> 99.999%) was injected into the bottle with an airtight syringe. At the same time, 5.0 ml of the sample was discharged from the bottle through the syringe to form headspace in the bottle. The bottle was then shaken for 30 min and balanced for 2 h at room temperature. A subsample of the equilibrated headspace was manually injected into a gas chromatograph (GC) with electron capture detection (SHIMADZU GC-2010 Plus), equipped with an HP-Plot/column (J and WGC Columns, Agilent Technologies, USA). The GC was calibrated daily with three different concentrations of standard gases (291, 617, and 4,980 ppbv N₂O/N₂, Research Institute of China National Standard Materials). The N₂O concentrations in the samples were calculated using the solubility function of Weiss and Price

(1980). The detection limit for N₂O analysis was 1.0 nmol L⁻¹, and the precision was ~2%.

Ancillary Measurements

Dissolved oxygen samples were transferred, stored, and analyzed using the Winkler method (Grasshoff et al., 1999). The relative standard deviation of the three samples was 2%. The pH of the collected samples was measured *in situ*. A Shimadzu TOC-L analyzer was used to repeat the sample three times in the laboratory, and the DIC concentrations of all repeat subsamples were measured. Water samples for nutrient analysis were filtered through 0.45- μm acetate cellulose membrane filters. The filtrates were poisoned with HgCl₂ and stored in the dark at 4°C. Nutrients, including ammonium (NH₄⁺), nitrate (NO₃⁻), and nitrite (NO₂⁻), were determined using a Technicon AA3 Auto-Analyzer (Bran+Luebbe) according to classical colorimetric methods (Wu et al., 2019). NH₄⁺, NO₃⁻, and NO₂⁻ were measured using the indophenol blue method, the

copper–cadmium column reduction method, and the phosphor-molybdate complex method, respectively.

Statistical Analysis

Repeated two-way multivariate ANOVA (two-way MANOVA) with IBM SPSS Statistics software (version 23.0) was used to examine the effects of treatments and their interactions on N₂O production during incubation. Pearson correlation coefficients were used to evaluate the relationships between N₂O production and relevant physical and chemical indicators. This analysis was conducted using the “corrplot” package (Simko, 2017) for R statistical software (version 4.0.3, R Core Team, 2020). ABT modeling was used to explain the relative influence of related parameters on N₂O production using the “gbmplus” package in R (De’ath, 2007). SEM was used to assess the relative direct and indirect impacts of ocean acidification and hypoxia on N₂O production (Alsterberg et al., 2013; Shi et al., 2016; Zhang et al., 2019; Wang et al., 2020). The SEM fit was evaluated using the chi-square test (χ^2), comparative fit index (CFI), root mean square error of approximation (RMSEM), and other parameters.

RESULTS

Vertical and Horizontal Profiles of Seawater Chemical Properties

The sea surface temperature (SST) was horizontally stable at ~28°C across the entire sampling area. Vertical temperature profiles showed an obvious cold water mass in the bottom layer of A3, A4, and A5, implying an obvious thermocline in the middle layer. Vertical density profiles closely mirrored those of temperature, implying that the latter was the dominant factor controlling the former. In contrast, the halocline was much weaker, and the bottom-surface difference in salinity was mostly <1 across the vertical profile. Chlorophyll *a* showed little vertical variation but increased toward the land, with a maximum concentration of 17.5 $\mu\text{g L}^{-1}$ at A1.

Vertical profiles of DO and pH showed similar stratification patterns, with the surface and bottom layers being uniform or having weak gradients, but the middle layer having rapid changes (Figure 2). DO and pH were higher in the surface layer than in the bottom layer. As for oxygen, A3, A4, and A5 were located in the oxygen minimum zone ($\text{O}_2 < 3.0 \text{ mg L}^{-1}$), with the bottom of A3 having the lowest oxygen concentration and highest N₂O concentration (37.5 nmol L^{-1}). High N₂O production was accompanied by ocean acidification and hypoxia. The maximum N₂O patterns were consistent with rapid changes in DO and pH.

Seawater Chemical Properties After Incubation

Seawater that received the Hyp and Acid treatments differed in pH, DIC, DO, particulate organic carbon and nitrogen [particulate organic carbon (POC) and particulate organic nitrogen (PON)], and various inorganic nitrogen forms (NH_4^+ , NO_2^- , and NO_3^-) after 64 h of incubation (Tables 2A,B). For the four treatment groups, the concentrations of seawater NH_4^+ -N and NO_3^- -N at Dongjiang port were four to five times higher than that at the Hongxing wharf. In addition, Hyp produced the

highest NH_4^+ -N and NO_3^- -N. For NH_4^+ -N, all treatment groups were higher than the ambient control (Con), but, for NO_3^- -N, all treatment groups performed the same except for Acid (Figure 3). Compared with the Con, DO in Hyp at the Dongjiang port and the Hongxing wharf reached 3.7 and 2.4 mgL^{-1} , respectively, while pH for that group at the Dongjiang port and the Hongxing wharf reached 7.69 and 7.71, respectively.

At the Dongjiang port, Hyp had a significant effect on DO ($p < 0.001$) and NH_4^+ -N ($p < 0.01$) concentrations, while Acid had a significant effect on pH, POC, and PON ($p < 0.05$). At the Hongxing wharf, Hyp had a significant effect on DO ($p < 0.001$) and NO_3^- -N ($p < 0.05$) concentrations, while Acid had a significant effect on pH and DIC concentrations ($p < 0.001$). Significantly, interactive effects between Acid and Hyp on pH, POC, and PON ($p < 0.05$) were identified by a two-way MANOVA.

N₂O Concentrations in Incubated Seawater Samples

After incubation, N₂O production showed strong variability between the different treatments (Figure 4). First, both Hyp and Acid treatment had significant effects on N₂O potential production from different anthropogenic nitrogen input regions. Hyp had a positive effect on N₂O potential production compared with Acid; the higher the anthropogenic nitrogen input, the greater was the effect. Relative to Con, Acid resulted in mean N₂O production, dropping from 2.72 to 1.34 nmol L^{-1} at the Dongjiang port and from 23.09 to 6.38 nmol L^{-1} at the Hongxing wharf, while the observed N₂O concentrations decreased by 50.77 and 72.38%, respectively. Also, relative to Con, Hyp resulted in mean N₂O production, increasing from 2.72 to 4.08 nmol L^{-1} at the Dongjiang port and from 23.09 to 110.78 nmol L^{-1} at the Hongxing wharf, while the observed N₂O concentrations increased by 49.72 and 278.68%, respectively (Figure 4, Table 3). Similar to Hyp, Hyp+Acid significantly increased N₂O potential production (compared with Con) from 2.72 to 3.17 nmol L^{-1} at the Dongjiang port and from 23.09 to 58.68 nmol L^{-1} at the Hongxing wharf, while the observed N₂O concentrations increased by 16.23 and 96.38%, respectively (Figure 4A). As expected, regions with high anthropogenic nitrogen inputs responded more to different treatments, mainly because these inputs increased substrate concentrations for nitrification and denitrification, and nitrogen utilization increased (Figure 4).

Relationships Between N₂O Concentration and Relative Parameters

Pearson’s correlation analysis showed significant variability between different anthropogenic nitrogen input seawater responses to different treatments (Figure 5). There was a significant positive correlation between N₂O concentration and NH_4^+ -N at the Dongjiang port ($p < 0.001$), indicating that the N₂O in this region was mainly produced by nitrification, which was consistent with results from the open ocean (Beman et al., 2011). Seawater pH and DO were positively and negatively correlated with N₂O concentration, respectively, consistent

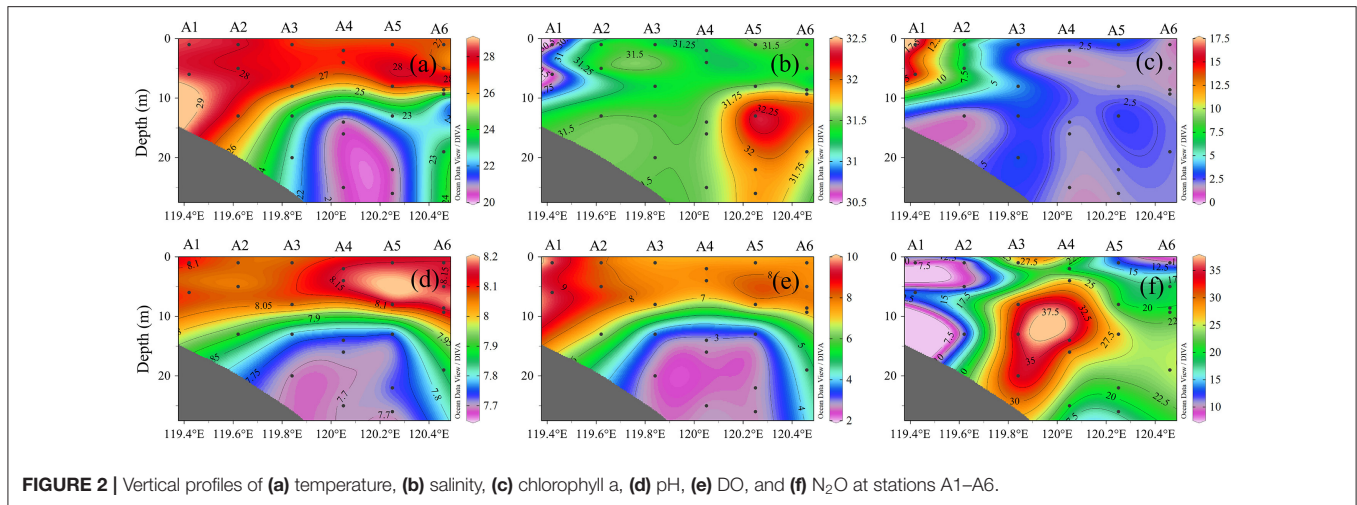


FIGURE 2 | Vertical profiles of (a) temperature, (b) salinity, (c) chlorophyll a, (d) pH, (e) DO, and (f) N₂O at stations A1–A6.

TABLE 2A | Effects of ocean acidification and hypoxia on chemical properties of seawater samples at the Dongjiang port.

Treatment	pH	DIC (mg L ⁻¹)	DO (mg L ⁻¹)	POC (mg L ⁻¹)	PON (mg L ⁻¹)	TN (mg L ⁻¹)	NH ₄ ⁺ (μmol L ⁻¹)	NO ₂ ⁻ (μmol L ⁻¹)	NO ₃ ⁻ (μmol L ⁻¹)	TIN (μmol L ⁻¹)
Con	7.91 ± 0.10	30.62 ± 0.26	8.7 ± 0.2	0.23 ± 0.04	0.04 ± 0.00	0.20 ± 0.04	2.44 ± 0.49	0.79 ± 0.21	10.71 ± 6.22	13.94 ± 6.72
Hyp	7.94 ± 0.01	29.51 ± 0.35	3.7 ± 0.5	0.35 ± 0.10	0.07 ± 0.02	0.20 ± 0.02	3.69 ± 0.46	0.78 ± 0.13	13.15 ± 3.72	17.58 ± 3.55
Acid	7.69 ± 0.03	31.35 ± 0.73	8.9 ± 0.1	0.23 ± 0.02	0.04 ± 0.00	0.20 ± 0.04	2.05 ± 0.88	0.88 ± 0.14	10.04 ± 1.65	12.96 ± 0.82
Acid+Hyp	7.76 ± 0.05	30.87 ± 1.57	3.7 ± 0.1	0.18 ± 0.00	0.03 ± 0.00	0.17 ± 0.03	2.99 ± 1.37	0.76 ± 0.14	11.78 ± 0.28	15.53 ± 1.28
F-value										
Hyp	0.30	2.40	1079.43***	1.68	1.43	0.80	4.64*	0.89	0.95	1.93
Acid	142.19***	4.09*	0.59	7.25**	7.96**	0.70	1.16	0.40	0.23	0.46
Hyp*Acid	5.34**	0.37	0.35	7.55**	6.78**	0.79	0.09	0.16	0.03	0.06

DIC, dissolved inorganic carbon; DO, dissolved oxygen; POC, particulate organic carbon; PON, particulate organic nitrogen; TN, total nitrogen; TIN, total inorganic nitrogen; Con, control treatment; Hyp, hypoxia treatment; Acid, ocean acidification treatment; and Acid+Hyp, the combination of hypoxia and ocean acidification treatment. *, **, and *** indicate significance levels at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

with our first hypothesis. However, at the Hongxing wharf, the results were different; there was a significant positive correlation between N₂O concentration and NO₃⁻-N ($p < 0.05$), and DO was significantly correlated with N₂O concentration ($p < 0.001$) (Figure 5). DO, pH, and DIC accounted for 31.3, 25.1, and 18.2%, respectively, of the relative influencing factors for N₂O potential production at the Dongjiang port much larger than for the other parameters. Similarly, DO, pH, and DIC accounted for 47.9, 17.5, and 14.5%, respectively, of the relative influencing factors for N₂O potential production at the Hongxing wharf (Figure 6). This result indicated that changes in DO, pH, and DIC might be responsible for the increase in N₂O potential production. Overall, hypoxia and acidification are likely to change the future patterns of ocean N₂O potential production.

The SEM also showed strong variability between different anthropogenic nitrogen input seawater responses to different treatments (Figure 7). The total effect of ocean acidification and hypoxia on seawater N₂O potential production at the Hongxing wharf was greater than at the Dongjiang port, which may be related to the NH₄⁺-N wastewater discharged by humans. These results showed that the overall effect of hypoxia on seawater N₂O potential production was positive, while ocean acidification was

negative, in agreement with our incubation experiments. SEM also confirmed the regulatory effect of inorganic nutrients on the effects of hypoxia and ocean acidification on seawater N₂O potential production.

DISCUSSION

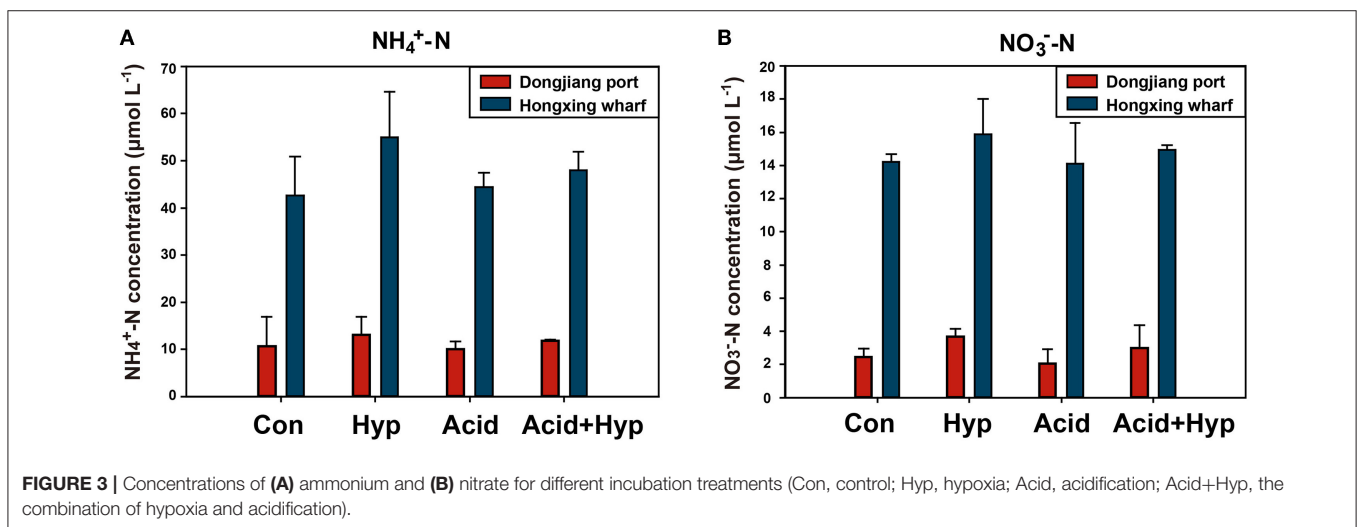
Potential Factors for Reducing N₂O Concentration From Ocean Acidification

Our results showed that CO₂-driven ocean acidification reduced N₂O potential production in seawater. In the polar Atlantic Ocean, N₂O production was sensitive to pH; when pH decreased by 0.06–0.4, N₂O production decreased by 2.4–44% (Rees et al., 2016). The generation of seawater N₂O under normal oxygen conditions has always been considered a product of nitrification (Beman et al., 2011). Previous studies suggested that ocean acidification would lead to a decrease in the ammonia oxidation rate, and reducing seawater pH also suppressed seawater N₂O emissions (Beman et al., 2011). Sample collection for the incubation experiments was poured from the closed water sampler and immediately kept in the dark, and we assumed that the sampling process and phytoplankton had little influence on

TABLE 2B | Effects of ocean acidification and hypoxia on water chemical properties at the Hongxing wharf.

Treatment	pH	DIC (mg L ⁻¹)	DO (mg L ⁻¹)	POC (mg L ⁻¹)	PON (mg L ⁻¹)	TN (mg L ⁻¹)	NH ₄ ⁺ (μmol L ⁻¹)	NO ₂ ⁻ (μmol L ⁻¹)	NO ₃ ⁻ (μmol L ⁻¹)	TIN (μmol L ⁻¹)
Con	7.90 ± 0.01	35.82 ± 0.26	6.7 ± 0.3	0.23 ± 0.05	0.05 ± 0.01	0.32 ± 0.16	14.20 ± 0.49	1.62 ± 0.15	42.67 ± 8.24	58.49 ± 8.83
Hyp	7.95 ± 0.04	35.21 ± 0.65	2.4 ± 0.2	0.40 ± 0.03	0.07 ± 0.01	0.39 ± 0.03	15.90 ± 2.13	1.53 ± 0.40	54.96 ± 9.74	72.38 ± 12.24
Acid	7.71 ± 0.02	37.74 ± 0.70	6.9 ± 0.2	0.37 ± 0.09	0.06 ± 0.02	0.38 ± 0.05	14.90 ± 2.46	1.83 ± 0.32	44.41 ± 3.05	60.35 ± 5.50
Acid+Hyp	7.69 ± 0.07	36.69 ± 0.50	2.2 ± 0.4	0.43 ± 0.14	0.07 ± 0.02	0.38 ± 0.03	14.93 ± 0.30	1.57 ± 0.15	47.93 ± 3.95	64.43 ± 4.37
F-value										
Hyp	0.21	6.67	726.19***	5.70*	4.15	0.47	1.74	1.23	3.99*	3.50*
Acid	93.70***	28.04***	0.00	2.73	1.25	0.23	0.31	0.64	0.45	0.40
Hyp*Acid	2.05	0.46	1.17	1.14	0.41	0.48	0.20	0.27	1.23	1.04

DIC, dissolved inorganic carbon; DO, dissolved oxygen; POC, particulate organic carbon; PON, particulate organic nitrogen; TN, total nitrogen; TIN, total inorganic nitrogen; Con, control treatment; Hyp, hypoxia treatment; Acid, ocean acidification treatment; and Acid+Hyp, the combination of hypoxia and ocean acidification treatment. *, and *** indicate significance levels at $p < 0.05$, and $p < 0.001$, respectively.



NH₃ or NH₄⁺. Since it has been shown that the composition of nitrifying bacterial communities can resist changes in ocean acidification, the observed N₂O concentration changes seem more likely to be related to changes in the NH₄⁺:NH₃ equilibrium (Hutchins et al., 2009; Beman et al., 2011). pH may play an indirect role in controlling N₂O potential production because it changes the chemical speciation of NH₄⁺:NH₃ covaries with other factors. NH₃ acts as a substrate for nitrification (Ward, 2008). According to the equilibrium equation of Bell et al. (2008), a decrease in seawater pH from 8.1 to 7.8 will lead to a 50% decrease in NH₃ concentration under constant temperature (22°C) and salinity (Wyatt et al., 2010). Using that equation, we estimated the NH₃ concentrations based on the calculated pKa values, pH, and NH₄⁺ concentrations measured *in situ*. A pKa fixed value of 9.25 suggested that NH₃ typically accounted for 6.3% of the total NHx. Beman et al. (2011) found significant correlations between the percentage changes in NH₃, pH, and ammonium oxidation rates (Hutchins et al., 2009) and (Kitidis et al., 2011) between pH and ammonium oxidation. We showed a similar relationship between the absolute values of pH and N₂O and the correlation between N₂O and NH₃ in the incubation

experiments (Figure 8) ($R^2 = 0.63$, $p < 0.05$) as a result of the induced shift in the NH₄⁺:NH₃ ratio. Our results indicated that changes in pH of 0.25 in the coastal waters of the Bohai Sea may lead to decreases in N₂O potential production of 50.77 and 72.38% due to a reduced NH₃ regime of 33.8 and 40.2%, respectively.

In contrast, Breider et al. (2019) examined the response of N₂O production from nitrification to acidification in a series of incubation experiments conducted in the subtropical and subarctic western North Pacific, showing that, when the pH decreased, the N₂O production rate significantly increased during the nitrification process, while the nitrification rate remained stable or decreased. We suggest two possible explanations for this difference. First, despite the beginning oxygen of Acid being comparable to the surrounding oxygen concentration, after 64 h, the oxygen centralization in the incubation bottles might have slightly diminished, hindering nitrification, on the other hand, according to Ji et al. (2018), when O₂ > 6 μmol L⁻¹ was 98% and showed complete inhibition on N₂O production from NO₂⁻ and NO₃⁻ reduction. The oxygen consumed by our incubation process is limited

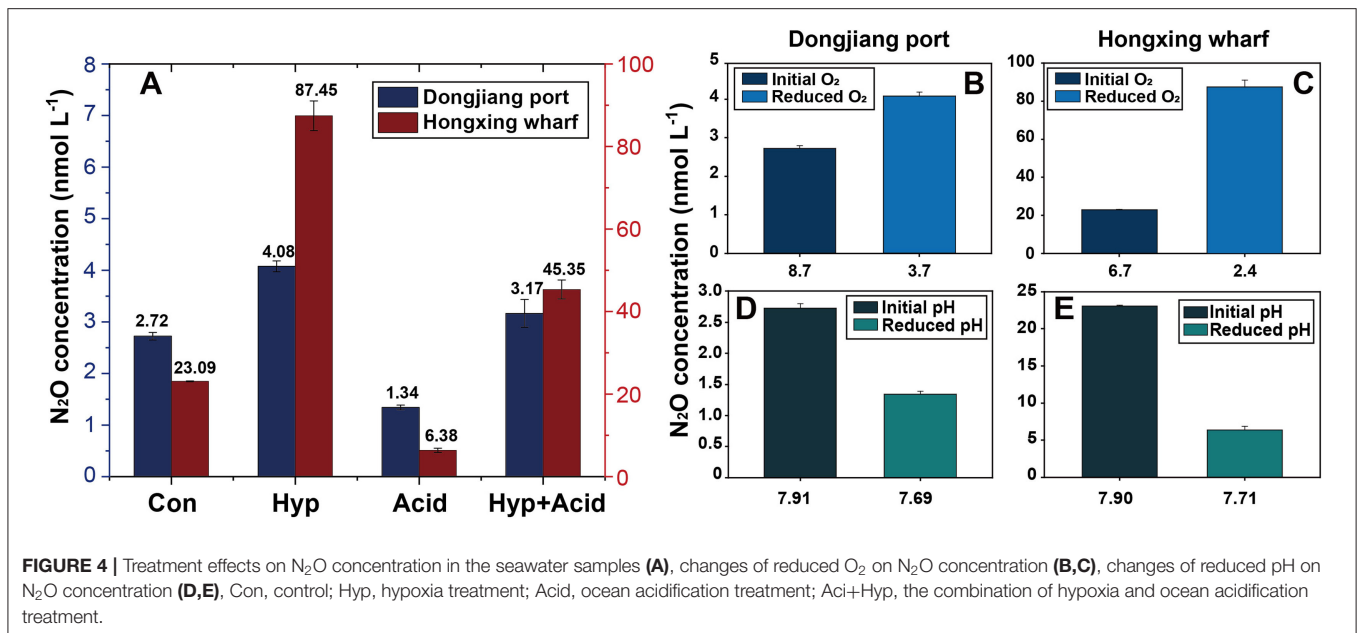


TABLE 3 | Increase in, and the increased rate of, N₂O under different treatments at the Dongjiang port and the Hongxing wharf.

Treatment	The increase of N ₂ O (nmol N ₂ O L ⁻¹)		The increase rate of N ₂ O(%)	
	Dongjiang port	Hongxing wharf	Dongjiang port	Hongxing wharf
Hyp	1.35	64.35	49.72	278.68
Acid	-1.38	-16.71	-50.77	-72.38
Acid+Hyp	0.44	22.26	16.23	96.38

Hyp, hypoxia; Acid, acidification; Aci+Hyp, the combination of hypoxia and acidification.

and does not make the oxygen in the incubation bottles reach the threshold value of denitrification, ultimately leading to a reduction in N₂O potential production. Second, the methods of acidification treatment were different between the two studies. They used HCl solutions and aerated the system with different concentrations of combined CO₂ and O₂ to achieve acidification (Breider et al., 2019). We changed the system pH and altered the seawater carbonate system, affecting N₂O potential production. In addition, pH reduction had a greater effect on N₂O in the anthropogenic nitrogen input samples, which may have been related to the high NH₄⁺-N in human wastewater discharge.

As ocean acidification intensifies, N₂O production decreases, indicating that future ocean acidification is likely to reduce ocean N₂O potential production, resulting in negative feedback relative to global warming (Beman et al., 2011). Beman et al. (2011) estimated that an observed reduction in nitrification rates of 3–44% would result in a reduction in global N₂O production of 0.06–0.83 Tg N/year over the next 20–30 years. Codispoti (2010) adopted a similar approach and assuming that 50% of the global ocean source of N₂O was 3.8 Tg Nyear⁻¹. For the less

anthropogenic nitrogen input area (pH decreased by 0.25), the estimated reduction of marine N₂O sources was ~0.96 year⁻¹. For the large anthropogenic nitrogen input area (pH decreased by 0.25), the estimated reduction of marine N₂O sources was ~1.38 year⁻¹.

Effect of Hypoxia on N₂O Potential Production

The potential production of N₂O increased significantly with decreasing DO concentration. Although the regulatory effect of oxygen on N₂O production in the ocean is widely accepted in principle [~100 papers cite Goreau et al. (1980) on N₂O production in the ocean], to the best of our knowledge, there have been few studies on the effect of oxygen on N₂O production in Bohai Bay. Due to eutrophication and environmental degradation, seasonal oxygen shortages in this location may expand in the future. Our results showed a non-linear relationship between N₂O concentration and O₂ concentration, with the former increasing as the latter decreases (Figure 9A). The basic linear relationship of ΔN₂O/AOU is often used to simulate the distribution of N₂O in the ocean, in which the slope value of ΔN₂O–AOU is regarded as the N₂O yield from nitrification, indicating the amount of N₂O produced per amount of O₂ consumed (Nevison et al., 2003). This theory varies widely (0.08–0.31 nmol N₂O per mmolO₂), making it difficult to capture the full dynamics of N₂O production in hypoxic water (Nevison et al., 2003). Trimmer et al. also proposed a mixed-effects model, which points out that, between 1 and 30 mmol L⁻¹ O₂ concentration, a decrease in O₂ concentration leads to a clear exponential increase in N₂O (Yoshida et al., 1984); this is comparable to our results.

Both high and low anthropogenic nitrogen input seawater under Hyp resulted in a decreased NO₂⁻/NO₃⁻ ratio after

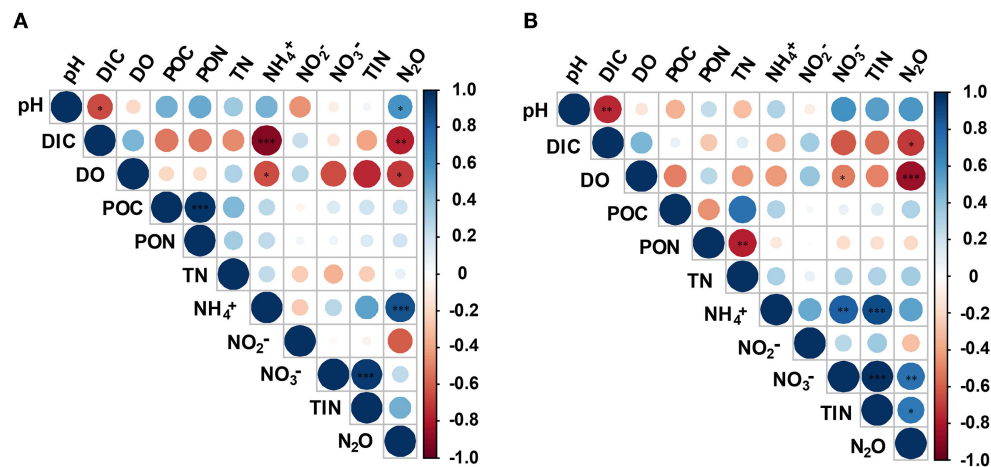


FIGURE 5 | Correlation analysis between environmental parameters and N₂O potential production for **(A)** the Dongjiang port and **(B)** the Hongxing wharf. Circle colors and sizes represent Pearson's correlation coefficients (*r*). *, **, and *** indicate significance levels at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. DIC, dissolved inorganic carbon; DO, dissolved oxygen; POC, particulate organic carbon; PON, particulate organic nitrogen; TN, total nitrogen; TIN, total inorganic nitrogen.

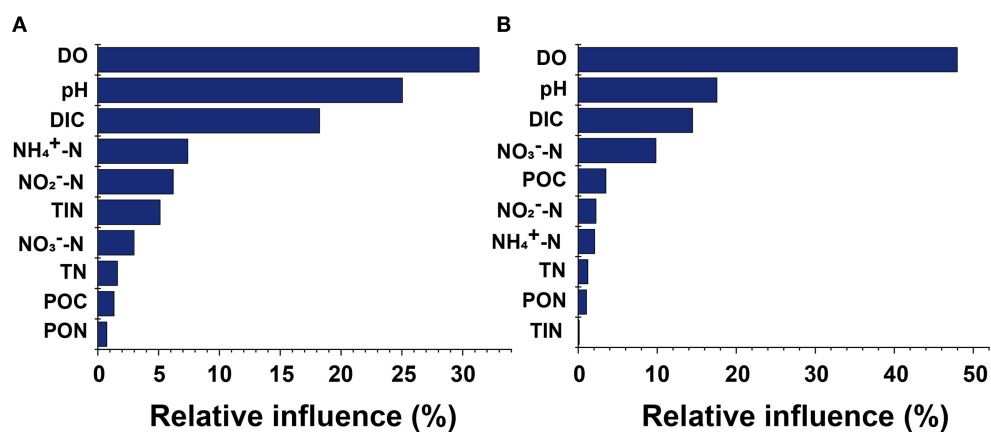


FIGURE 6 | Relative influences of parameter contributions to seawater N₂O potential production for **(A)** the Dongjiang port and **(B)** the Hongxing wharf. DIC, dissolved inorganic carbon; DO, dissolved oxygen; POC, particulate organic carbon; PON, particulate organic nitrogen; TN, total nitrogen; TIN, total inorganic nitrogen.

incubation (**Figure 9B**). We speculate that nitrification and denitrification may occur together with a decrease in oxygen concentration (even when the oxygen concentration does not reach complete denitrification), and as NO₂⁻ is the basis for both nitrification and denitrification (Wilson et al., 2014), the consumption of NO₂⁻ is greater than production. This is similar to Ji et al. (2018), whose ¹⁵N tracer incubation experiments showed that denitrification was the dominant source of N₂O production in the anoxic and the peripheral suboxic waters, because rates of N₂O production from NO₂⁻ and NO₃⁻ reduction were 10- to 100-fold higher than N₂O production from NH₄⁺ oxidation. Our results also support this view, indicating that oxygen concentration in the oxycline supports nitrification and denitrification, resulting in maximum N₂O concentration.

Direct vs. Indirect Effects of Ocean Acidification and Hypoxia on Inorganic Nitrogen and N₂O Potential Production

Our second hypothesis was that hypoxia and ocean acidification changed N₂O potential production by altering seawater properties rather than directly affecting N₂O production. DO, pH, and DIC were the key factors affecting N₂O potential production (**Figure 6**). SEM also showed strong variability among different anthropogenic nitrogen input seawater responses to different treatments. These results suggested a role for the indirect effects of ocean acidification and hypoxia on inorganic nitrogen and N₂O potential production in coastal marine systems. Ocean acidification may directly or indirectly affect N₂O potential production through a variety

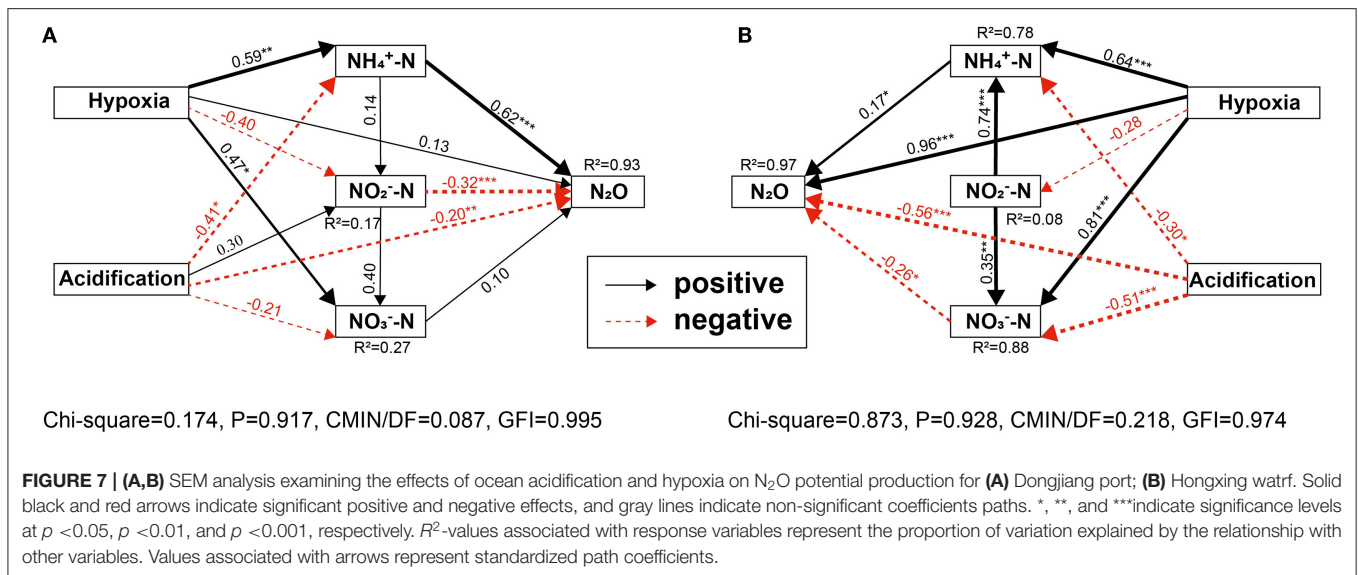


FIGURE 7 | (A,B) SEM analysis examining the effects of ocean acidification and hypoxia on N₂O potential production for **(A)** Dongjiang port; **(B)** Hongxing wharf. Solid black and red arrows indicate significant positive and negative effects, and gray lines indicate non-significant coefficients paths. *, **, and *** indicate significance levels at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. R^2 -values associated with response variables represent the proportion of variation explained by the relationship with other variables. Values associated with arrows represent standardized path coefficients.

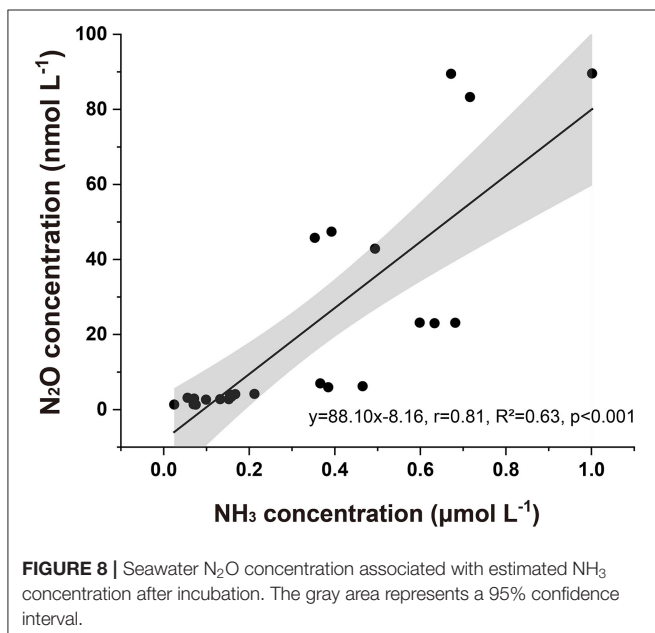


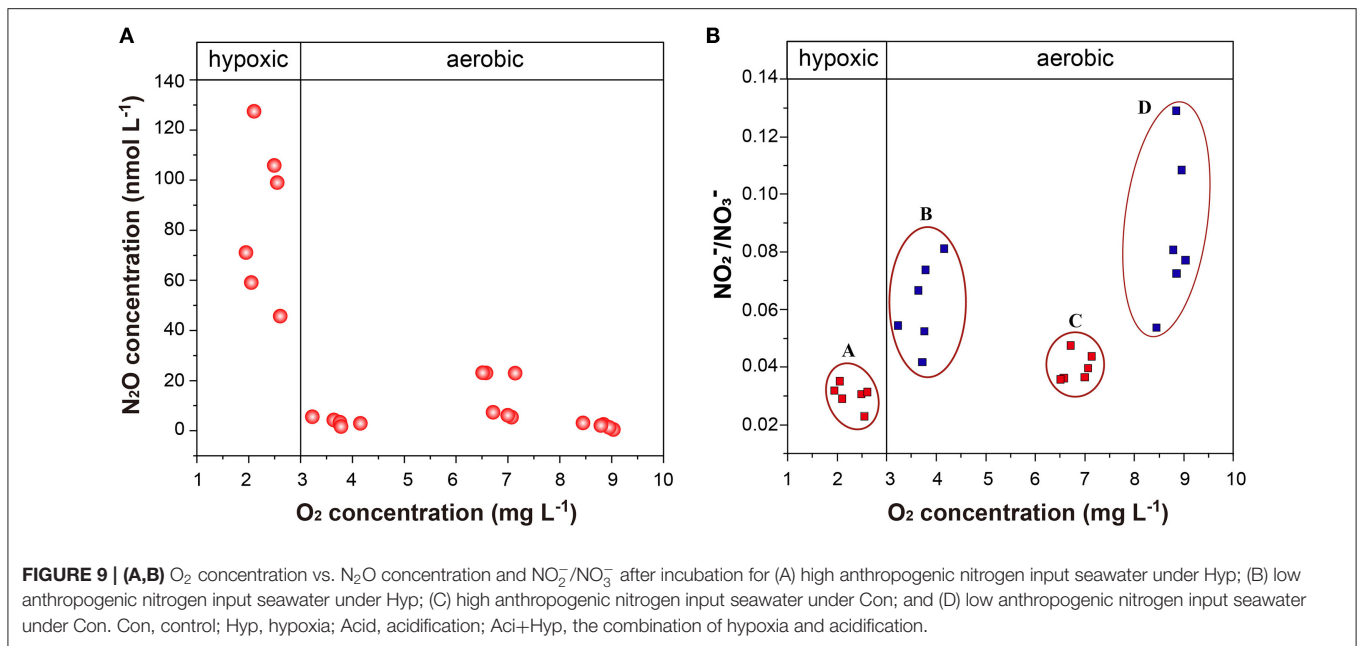
FIGURE 8 | Seawater N₂O concentration associated with estimated NH₃ concentration after incubation. The gray area represents a 95% confidence interval.

of mechanisms, including AOA abundance (Rees et al., 2016) and nitrification rates (Beman et al., 2011). In the Dongjiang port, both hypoxia and ocean acidification had direct effects on seawater N₂O potential production (Hypoxia → N₂O, path coefficients >0.10, but $P < 0.05$; acidification → N₂O, path coefficients >0.10 and $P > 0.05$) but indirect effects *via* NH₄⁺-N, NO₂⁻-N, and NO₃⁻-N (**Figure 9A**). Hypoxia increased NH₄⁺-N and NO₃⁻-N, which, in turn, increased N₂O potential production, while hypoxia also reduced NO₂⁻-N and increased N₂O potential production. Thus, the total effect of hypoxia on seawater N₂O potential production was positive (0.67). Ocean acidification reduced NH₄⁺-N and NO₃⁻-N, which, in turn, reduced N₂O potential production, and ocean acidification also

increased NO₂⁻-N and reduced N₂O potential production. Thus, the total effects of ocean acidification on seawater N₂O potential production were negative (0.57). Hypoxia increased, whereas ocean acidification reduced, seawater N₂O potential production via direct effects (**Figure 9A**).

Interestingly, the direct and indirect effects of hypoxia treatment on changes in N₂O potential production counteracted the effects of ocean acidification. As hypoxia and ocean acidification increased N₂O potential production, the negative effects of ocean acidification were canceled, and the total effects were positive (0.10). The situation seems to have changed at the Hongxing wharf, where both hypoxia and ocean acidification had significant direct effects on seawater N₂O potential production (Hypoxia → N₂O, path coefficients >0.10 and $P < 0.05$; acidification → N₂O, path coefficients >0.10 and $P > 0.05$), with indirect effects *via* NO₃⁻-N (**Figure 9B**). Hypoxia increased NH₄⁺-N and NO₃⁻-N, which, in turn, increased N₂O potential production. Thus, the total effect of hypoxia on seawater N₂O potential production was positive (0.86). Ocean acidification reduced NH₄⁺-N and NO₃⁻-N, which, in turn, reduced N₂O potential production. Thus, the total effects of ocean acidification on seawater N₂O potential production were negative (0.48). Ocean acidification reduced NH₄⁺-N and NO₃⁻-N, which, in turn, reduced N₂O potential production. Hypoxia increased, whereas ocean acidification reduced seawater N₂O potential production *via* direct effects (**Figure 9B**). Interestingly, the direct and indirect effects of hypoxia treatment on changes in N₂O potential production counteracted the effects of ocean acidification. As hypoxia and ocean acidification increased N₂O potential production, the negative effects of ocean acidification were canceled, and the total effects were positive (0.38).

The causes of hypoxia and acidification at the bottom of the Bohai Sea are very complex, including the coupling of physical, chemical, and biological processes, the most important of which is caused by the oxygen consumption of biological processes.



It is well-known that DO in seawater is significantly related to photosynthesis and the decomposition of organic matter in phytoplankton. The photosynthesis of phytoplankton within the euphotic layer results in high dissolved oxygen. *In situ* biological production, especially that driven by nitrification, is commonly considered to be the major mechanism of N₂O production in the ocean (Naqvi et al., 2010). The formation of a seawater thermocline limits the exchange of DO from the surface to the bottom, a key physical mechanism of the oxycline in the Bohai Sea. Seasonal seawater stratification prevents the bottom N₂O from being transported upward, such that nitrification and denitrification occur together, leading to the accumulation of N₂O. Generally, phytoplankton blooms occur in spring with increasing temperature and light intensity in the Bohai Sea. The concentration of chlorophyll a reaches its highest level in summer with coefficients sunlight and terrestrial nutrient input (Liu and Yin, 2010). Therefore, due to the propagation of phytoplankton in nutrient-rich layers from spring to summer, the deposition of organic matter and aerobic decomposition provides the basis for hypoxia occurrence at the bottom during summer. This limitation of oxygen supply further increases the pressure of bottom mineralization, exacerbating bottom hypoxia and leading to denitrification. Aerobic decomposition of organic matter increases the pCO₂ of the underlying seawater and increases acidification. Our experimental results showed that ocean acidification can attenuate N₂O potential production in the OMZ, which may also be an important reason for the lower concentration of N₂O at the bottom layer than in the middle layer.

Our experiments also showed that the coupling of ocean acidification and hypoxia increased N₂O production in coastal water columns. The decrease in oxygen levels (Gruber, 2011; Gattuso et al., 2015) and the decrease in organic matter output to

the deep sea due to ocean acidification (Gehlen et al., 2011, due to reduction in ballast effects) both affect N₂O generation and atmospheric release. Hypoxia stimulates seawater N₂O potential production with positive feedback effects on global warming, and ocean acidification weakens the increase in N₂O caused by hypoxia. Previous studies argued that the occurrence of seasonal hypoxia in the Bohai Sea is often accompanied by ocean acidification (Riebesell and Gattuso, 2015; Wei et al., 2019; Zhai et al., 2019; Song et al., 2020), but the results show that hypoxia and ocean acidification are antagonistic to each other and that artificial nitrogen input can amplify this change. This finding provides a basis for more accurate estimations of offshore greenhouse gas production models.

CONCLUSIONS

Ocean acidification and hypoxia decreased and increased N₂O potential production, respectively. Incubation experiments showed that the combination of ocean acidification and hypoxia significantly increased N₂O potential production, but, individually, there was an antagonistic relationship between the two. Seawater pH, DIC, DO, NH₄⁺-N, and TIN were the primary predictors of sensitivity to N₂O potential production. SEM analysis showed that the direct and indirect effects of hypoxia treatment on N₂O potential production changes counteracted the effects of ocean acidification treatment. As hypoxia and ocean acidification together increased N₂O potential production, the negative effects of ocean acidification were canceled out, resulting in overall positive effects. Generally speaking, our results showed that N₂O potential production from coastal waters of the Bohai Sea may increase under future climate change scenarios by enhancing both ocean acidification and hypoxia. Future research

should focus on whether these experimental results could be extrapolated to directly assess the response between *in situ* N₂O potential production and climate change. Moreover, further attention should be paid to areas with high anthropogenic nitrogen input.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

JS designed the study. TG and DJ performed the experiments. TG, DJ, XM, LP, YW and JS analyzed the data. GZ provided the nutrient data. TG, DJ and JS wrote the manuscript with input from all authors.

REFERENCES

- Alsterberg, C., Eklof, J. S., Gamfeldt, L., Havenhand, J. N., and Sundback, K. (2013). Consumers mediate the effects of experimental ocean acidification and warming on primary producers. *Proc. Natl. Acad. Sci. U.S.A.* 110, 8603–8608. doi: 10.1073/pnas.1303797110
- Arévalo-Martínez, D. L., Kock, A., Löscher, C. R., Schmitz, R. A., and Bange, H. W. (2015). Massive nitrous oxide emissions from the tropical South Pacific Ocean. *Nat. Geosci.* 8, 530–533. doi: 10.1038/ngeo2469
- Babbin, A. R., Bianchi, D., Jayakumar, A., and Ward, B. B. (2015). Rapid nitrous oxide cycling in the suboxic ocean. *Science* 348, 1127–1129. doi: 10.1126/science.aaa8380
- Bange, H. W. (2006). New directions: the importance of oceanic nitrous oxide emissions. *Atmos. Environ.* 40, 198–199. doi: 10.1016/j.atmosenv.2005.09.030
- Bell, T. G., Johnson, M. T., Jickells, T. D., and Liss, P. S. (2008). Ammonia/ammonium dissociation coefficient in seawater: a significant numerical correction. *Environ. Chem.* 4, 183–186. doi: 10.1071/EN07032
- Beman, J. M., Chow, C. E., King, A. L., Feng, Y., and Fuhrman, J. A. (2011). Global declines in oceanic nitrification rates as a consequence of ocean acidification. *Proc. Natl. Acad. Sci. U.S.A.* 108, 208–213. doi: 10.2307/25770752
- Breider, F., Yoshikawa, C., Makabe, A., Toyoda, S., Wakita, M., Matsui, Y., et al. (2019). Response of N₂O production rate to ocean acidification in the western North Pacific. *Nat. Clim. Change* 9, 954–958. doi: 10.1038/s41558-019-0605-7
- Codispoti, L. A. (2010). Interesting times for marine N₂O. *Science* 327, 1339–1340. doi: 10.1126/science.1184945
- Crutzen, P. J., and Ehhalt, D., (1977). Effects of nitrogen fertilizers and combustion on the stratospheric ozone layer. *Ambio*, 6, 112–117.
- De'ath, G. (2007). Boosted trees for ecological modeling and prediction. *Ecology* 88, 243–251. doi: 10.2307/27651085
- Freing, A., Wallace, D., and Bange, H. W. (2012). Global oceanic production of nitrous oxide. *Philos. Trans. R. Soc. B Biol. Sci.* 367, 1245–1255. doi: 10.1098/rstb.2011.0360
- Gattuso, J.-P., Magnan, A., Bille, R., Cheung, W. W. L., and Howes, E. L. (2015). Contrasting futures for ocean and society from different anthropogenic CO₂ emissions scenarios. *Science* 349, 4722–4722. doi: 10.1126/science.aac4722
- Gehlen, M., Gruber, N., and Skalander, R. (2011). Biogeochemical consequences of ocean acidification and feedbacks to the earth system. doi: 10.1093/oso/9780199591091.003.0017
- Goreau, T. J., Kaplan, W. A., Wofsy, S. C., Mcelroy, M. B., and Watson, S. W. (1980). Production of NO₂⁻ and N₂O by nitrifying bacteria at

FUNDING

This research was financially supported by the National Key Research and Development Project of China (2019YFC1407803), the National Natural Science Foundation of China (42006174 and 41876134). This work was also supported by the Changjiang Scholar Program of Chinese Ministry of Education (T2014253) to JS and the Scientific Research Project of Tianjin Municipal Education Commission (2017KJ012) and the Open Fund of Tianjin Key Laboratory of Marine Resources and Chemistry (201901) to DJ and Key Laboratory of Integrated Marine Monitoring and Applied Technologies for Harmful Alga Blooms Open Project (MATHAB201829).

ACKNOWLEDGMENTS

We are grateful to all laboratory colleagues for their help with this study and the writing of this paper.

- reduced concentrations of oxygen. *Appl. Environ. Microbiol.* 40, 526–532. doi: 10.1016/0141-4607(80)90006-2
- Grasshoff, K., Kremling, K., and Ehrhardt, M. (1999). *Methods of Seawater Analysis*. Weinheim: Verlag Chemie. doi: 10.1002/9783527613984
- Gruber, N. (2011). Warming up, turning sour, losing breath: ocean biogeochemistry under global change. *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.* 369, 1980–1996. doi: 10.1098/rsta.2011.0003
- Hutchins, D. A., Fu, F.-X., Zhang, Y., Warner, M. E., Feng, Y., Portune, J. K., et al. (2007). CO₂ control of Trichodesmium N₂ fixation, photosynthesis, growth rates, and elemental ratios: implications for past, present, and future ocean biogeochemistry. *Limnol. Oceanogr.* 52, 1293–1304. doi: 10.4319/lo.2007.52.4.1293
- Hutchins, D. A., Mulholland, M. R., and Fu, F. X. (2009). Nutrient cycles and marine microbes in a CO₂-enriched ocean. *Oceanography* 22, 128–145. doi: 10.5670/oceanog.2009.103
- Ji, Q., Babbin, A. R., Jayakumar, A., Oleynik, S., and Ward, B. B. (2015). Nitrous oxide production by nitrification and denitrification in the Eastern Tropical South Pacific oxygen minimum zone. *Geophys. Res. Lett.* 42, 10755–10764. doi: 10.1002/2015gl066853
- Ji, Q., Buitenhuis, E., Suntharalingam, P., Sarmiento, J. L., and Ward, B. B. (2018). Global nitrous oxide production determined by oxygen sensitivity of nitrification and denitrification. *Glob. Biogeochem. Cycles* 32, 1790–1802. doi: 10.1029/2018gb005887
- Kalvelage, T., Lavik, G., Lam, P., Contreras, S., Arteaga, L., Löscher, C. R., et al. (2013). Nitrogen cycling driven by organic matter export in the South Pacific oxygen minimum zone. *Nat. Geosci.* 6, 228–234. doi: 10.1038/NNGEO1739
- Kitidis, V., Laverock, B., McNeill, L. C., Beesley, A., Cummings, D., Tait, K., et al. (2011). Impact of ocean acidification on benthic and water column ammonia oxidation. *Geophys. Res. Lett.* 38:21603. doi: 10.1029/2011GL049095
- Kock, A., Arévalo-Martínez, D. L., Löscher, C. R., and Bange, H. W. (2016). Extreme N₂O accumulation in the coastal oxygen minimum zone off Peru. *Biogeosciences* 13, 827–840. doi: 10.5194/bg-13-827-2016
- Le Quéré, C., Peters, G. P., Andres, R. J., Andrew, R. M., Boden, T., Ciais, P., et al. (2014). Global carbon budget 2013. *Earth Syst. Sci. Data Discuss.* 6, 689–760. doi: 10.5194/essdd-6-689-2013
- Li, Y., Wolanski, E., and Zhang, H. (2015). What processes control the net currents through shallow straits? A review with application to the Bohai Strait, China. *Estuar. Coast. Shelf Sci.* 158, 1–11. doi: 10.1016/j.ecss.2015.03.013
- Lin, X., Xie, S.-P., Chen, X., and Xu, L. (2006). A well-mixed warm water column in the central Bohai Sea in summer: effects of tidal and surface wave mixing. *J. Geophys. Res.* 111:C11. doi: 10.1029/2006jc003504

- Liu, H., and Yin, B. (2010). Numerical investigation of nutrient limitations in the Bohai Sea. *Mar. Environ. Res.* 70, 308–317. doi: 10.1016/j.marenvres.2010.06.003
- Naqvi, S. W. A., Bange, H. W., Fariás, L., Monteiro, P. M. S., Scranton, M. I., and Zhang, J. (2010). Marine hypoxia/anoxia as a source of CH₄ and N₂O. *Biogeosciences* 7, 2159–2190. doi: 10.5194/bg-7-2159-2010
- Naqvi, S. W. A., Jayakumar, D. A., Narvekar, P. V., Naik, H., Sarma, V. V., D'Souza, W., et al. (2000). Increased marine production of N₂O due to intensifying anoxia on the Indian continental shelf. *Nature* 408, 346–349. doi: 10.1038/35042551
- Naqvi, S. W. A., Yoshinari, T., Jayakumar, D. A., Altabet, M. A., Narvekar, P. V., Devol, A. H., et al. (1998). Budgetary and biogeochemical implications of N₂O isotope signatures in the Arabian Sea. *Nature* 394, 462–464. doi: 10.1038/28828
- Nevison, C., Butler, J. H., and Elkins, J. W. (2003). Global distribution of N₂O and the ΔN₂O-AOU yield in the subsurface ocean. *Glob. Biogeochem. Cycles* 17, 1119. doi: 10.1029/2003GB002068
- Nevison, C. D., Weiss, R. F., and Erickson, D. J. III. (1995). Global oceanic emissions of nitrous oxide. *J. Geophys. Res. Oceans* 100, 15809–15820. doi: 10.1029/95jc00684
- Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., et al. (2005). Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* 437, 681–686. doi: 10.1038/nature04095
- Qin, D. H. (2014). Climate change science and sustainable development. *Prog. Geogr.* 33, 874–883. doi: 10.11820/dlkxjz.2014.07.002
- R Core Team (2020). *R: A Language and Environment for Statistical Computing*. Vienna.
- Rees, A. P., Brown, I. J., Jayakumar, A., and Ward, B. B. (2016). The inhibition of N₂O production by ocean acidification in cold temperate and polar waters. *Deep Sea Res. II Top. Stud. Oceanogr.* 127, 93–101. doi: 10.1016/j.dsr2.2015.12.006
- Riebesell, U., and Gattuso, J. P. (2015). Lessons learned from ocean acidification research. *Nat. Clim. Change* 5, 12–14. doi: 10.1038/nclimate2456
- Shi, Z., Xu, X., Souza, L., Wilcox, K., Jiang, L., Xia, J., et al. (2016). Dual mechanisms regulate ecosystem stability under decade-long warming and hay harvest. *Nat. Commun.* 7:11973. doi: 10.1038/ncomms11973
- Simko, T. W. (2017). *R Package "Corrplot": Visualization of a Correlation Matrix*.
- Song, G., Zhao, L., Chai, F., Liu, F., Li, M., Xie, H., et al. (2020). Summertime oxygen depletion and acidification in Bohai sea, China. *Front. Mar. Sci.* 7:252. doi: 10.3389/fmars.2020.00252
- Stocker, T. F., Qin, D., Plattner, G. K., Tignor, M., Allen, S. K., and Boschung, J., et al. (2013). *The Physical Science Basis*. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Computational Geometry.
- Trimmer, M., Chronopoulou, P.-M., Maanoja, S. T., Upstill-Goddard, R. C., Kitidis, V., and Purdy K. J. (2016). Nitrous oxide as a function of oxygen and archaeal gene abundance in the North Pacific. *Nat. Commun.* 7:13451. doi: 10.1038/ncomms13451
- Wan, R., Chen, Y., Zheng, X., Su, Y., and Li, M. (2016). Effect of CO₂ on microbial denitrification via inhibiting electron transport and consumption. *Environ. Sci. Technol.* 50, 9915–9922. doi: 10.1021/acs.est.5b05850
- Wang, J., Cui, W., Che, Z., Liang, F., Wen, Y., Zhan, M., et al. (2020). Effects of synthetic nitrogen fertilizer and manure on fungal and bacterial contributions to N₂O production along a soil acidity gradient. *Sci. Tot. Environ.* 753:142011. doi: 10.1016/j.scitotenv.2020.142011
- Ward, B. (2008). "Nitrification in marine systems," in *Nitrogen in the Marine Environment, 2nd Edn*, eds G. C. Douglas, A. B. Deborah, R. M. Margaret, and J. C. Edward (Academic Press) 199–261. doi: 10.1016/B978-0-12-372522-6.00005-0
- Wei, Q., Wang, B., Yao, Q., Xue, L., Sun, J., Xin, M., et al. (2019). Spatiotemporal variations in the summer hypoxia in the Bohai Sea (China) and controlling mechanisms. *Mar. Pollut. Bull.* 138, 125–134. doi: 10.1016/j.marpollbul.2018.11.041
- Weiss, R. F., and Price, B. A. (1980). Nitrous oxide solubility in water and seawater. *Mar. Chem.* 8, 347–359. doi: 10.1016/0304-4203(80)90024-9
- Wilson, S. T., del Valle, D. A., Segura-Noguera, M., and Karl, D. M. (2014). A role for nitrite in the production of nitrous oxide in the lower euphotic zone of the oligotrophic North Pacific Ocean. *Deep Sea Res. I Oceanogr. Res. Pap.* 85, 47–55. doi: 10.1016/j.dsr.2013.11.008
- Wu, C., Kan, J., Liu, H., Pujari, L., Guo, C., Wang, X., et al. (2019). Heterotrophic bacteria dominate the diazotrophic community in the Eastern Indian Ocean (EIO) during Pre-Southwest Monsoon. *Microb. Ecol.* 78, 804–819. doi: 10.1007/s00248-019-01355-1
- Wyatt, N. J., Kitidis, V., Woodward, E. M. S., Rees, A. P., Widdicombe, S., and Lohan, A. M. (2010). Effects of high CO₂ on the fixed nitrogen inventory of the Western English Channel. *J. Plank. Res.* 32, 631–641. doi: 10.1093/plankt/fbp140
- Yoshida, N., Hattori, A., Saino, T., Matsuo, S., and Wada, E. (1984). ¹⁵N/¹⁴N ratio of dissolved N₂O in the eastern tropical Pacific Ocean. *Nature* 307, 442–444. doi: 10.1038/307442a0
- Zhai, W., Zhao, H., Su, J., Liu, P., Li, Y., Zheng, N., et al. (2019). Emergence of summertime hypoxia and concurrent carbonate mineral suppression in the central Bohai sea, China. *J. Geophys. Res. Biogeosci.* 124, 2768–2785. doi: 10.1029/2019JG005120
- Zhai, W., Zhao, H., Zheng, N., and Xu, Y. (2012). Coastal acidification in summer bottom oxygen-depleted waters in northwestern–northern Bohai Sea from June to August in 2011 (in Chinese). *Chin Sci Bull.* 57, 753–758. doi: 10.1007/s11434-011-4949-2
- Zhang, H., Li, Y., Tang, C., Zou, T., Yu, J., and Guo, K. (2016). Spatial characteristics and formation mechanisms of bottom hypoxia zone in the Bohai Sea during summer (in Chinese). *Chin Sci Bull.* 61, 1612–1620. doi: 10.1360/N972015-00915
- Zhang, Y., Zhang, N., Yin, J., Yang, F., Zhao, Y., Jiang, Z., et al. (2019). Combination of warming and N inputs increases the temperature sensitivity of soil N₂O emission in a Tibetan alpine meadow. *Sci. Tot. Environ.* 704:135450. doi: 10.1016/j.scitotenv.2019.135450
- Zhou, F., Huang, D., and Su, J. (2009). Numerical simulation of the dual-core structure of the Bohai Sea cold bottom water in summer (in Chinese). *Sci. Bull.* 54, 4520–4528. doi: 10.1007/s11434-009-0019-4

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Gu, Jia, Ma, Peng, Zhang, Wei, Lou and Sun. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.