Warming Increases Nitrous Oxide Emission from the Littoral Zone of Lake Poyang, China

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Abstract: Littoral wetlands are globally important for sustainable development; however, they have recently been identified as critical hotspots of nitrous oxide (N₂O) emissions. N₂O flux from subtropical littoral wetlands remains unclear, especially under the current global warming environment. In the littoral zone of Lake Poyang, a simulated warming experiment was conducted to investigate N₂O flux. Open-top chambers were used to raise temperature, and the static chamber-gas chromatograph method was used to measure N₂O flux. Results showed that the littoral zone of Lake Poyang was an N₂O source, with an average flux rate of 8.9 µg N₂O m⁻² h⁻¹. Warming significantly increased N₂O emission (13.8 µg N₂O m⁻² h⁻¹ under warming treatment) by 54% compared to the control treatment. N₂O flux in the spring growing season was also significantly higher than that of the autumn growing season. In addition, temperature was not significantly related to N₂O flux, while soil moisture only explained about 7% of N₂O variation. These results imply that N₂O emission experiences positive feedback effect on the ongoing warming of the climate, and abiotic factors (e.g., soil temperature and soil moisture) were not main controls on N₂O variation in this littoral wetland.

Keywords: nitrous oxide; climate warming; littoral wetland

1. Introduction

The global temperature has increased by 0.85 °C over the past three decades and is projected to increase by 0.3–4.8 °C by the end of the 21st century [1]. Climate warming may exert great influences on greenhouse gas (GHG) fluxes in wetlands [2,3] due to the large carbon and nitrogen stocks/emissions and the high temperature sensitivity [4–6]. Nitrous oxide (N₂O) is a long-lived GHG, a dominant ozone-depleting substance, and an environmental air pollutant, which not only contributes to climate change but also impacts human health [7,8]. Although carbon dioxide (CO₂) is the most abundant GHG in the atmosphere, the global warming potential of N₂O is approximately 265 times higher than that of CO₂ over a 100-year timescale [9]. The concentration of atmospheric N₂O reached new highs in 2018 at 331 ppb, and natural sources (e.g., wetlands, forests, and oceans) contributed about 60% of global N₂O emissions [10]. However, the global N₂O budget is far from being fully understood due to the lack of available data from all natural ecosystems [11], especially in the largely unexplored littoral wetlands.

The impacts of climate warming on N₂O flux are complex, with positive [12], negative [13], or neutral [14] effects having been reported in previous studies. This is because warming may affect multiple processes, such as the characteristics of soil and microbial activities, which may further alter...
the pristine balance of N$_2$O production and consumption [12–16]. For example, Chen et al. (2017) found that warming could transform an alpine swamp meadow from an N$_2$O sink to an N$_2$O source [15]; Qiu et al. (2018) reported that warming can stimulate N$_2$O emission by increasing soil labile carbon, soil nitrogen mineralization, and the biomass of N$_2$O-producing microbes [16]. Moreover, soil temperature and soil moisture are two key factors affecting the dynamics of GHG and are highly sensitive to climate warming [17,18]. Experimental warming usually tends to increase soil temperature and decrease soil moisture and thereby stimulates N$_2$O emission by increasing available nitrogen and microbial activities [19,20]. However, another study has shown that warming has no impact on N$_2$O flux due to the uncertain effects of warming on soil moisture in different periods [21]. These inconsistent results indicate the inconclusive results regarding the response of N$_2$O flux to the soil environment under warming treatment. Therefore, we need more in-depth studies regarding the dependency of N$_2$O flux on soil temperature and soil moisture to improve our understanding of the N$_2$O cycle in wetlands under a future warming environment.

To date, most studies of the N$_2$O cycle have been conducted in cropland [22], grassland [23], or forest [24] ecosystems as well as boreal wetlands [25] rather than in subtropical or tropical wetlands. Littoral wetlands usually act as biogeochemically active transition zones between terrestrial and aquatic ecosystems [26] and emit more N$_2$O than pelagic areas from lakes [27]. Vegetated littoral wetlands are reported to be the hotspots of N$_2$O emission, with mean N$_2$O flux rate of 284 $\mu$g N$_2$O m$^{-2}$ h$^{-1}$ across China’s lakes [27]. Lake Poyang, the largest subtropical freshwater lake in China, includes a littoral zone and pelagic area. With the seasonal fluctuation of water level, the littoral zone of Lake Poyang varies between the inundated condition and the inundated condition and thus experiences dramatic variability in its biogeochemical cycle [28,29]. To our knowledge, previous studies of GHG fluxes are still insufficient in Lake Poyang. These studies have mainly focused on the temporal variations of plant-mediated carbon (CO$_2$ and CH$_4$) fluxes in this littoral wetland [30–32], but available data of N$_2$O flux are scarce, and the response of N$_2$O flux to climate warming also remains unknown. These unbalanced and inadequate studies urge us to assess N$_2$O emission from the perspective of this subtropical littoral wetland, especially with the consideration of the effect of current climate warming.

To address these knowledge gaps, we conducted a simulated warming experiment in the littoral zone of Lake Poyang during the uninundated period. We hypothesised that (1) the littoral zone of Lake Poyang is an N$_2$O source during the uninundated period, (2) warming has a positive effect on N$_2$O emission in this littoral wetland, and (3) soil temperature and soil moisture are the main controls on N$_2$O flux in the littoral zone of Lake Poyang during the uninundated period.

2. Materials and Methods

2.1. Study Site

This study was conducted in the littoral zone of Lake Poyang (29°27′00″ N, 116°01′14″ E), the largest subtropical freshwater lake in China. Located in a subtropical monsoon climatic zone, this region’s annual precipitation ranges from 1566 mm to 1847 mm, and annual mean air temperature ranges from 15 °C to 18 °C [29]. Lake Poyang is an ephemeral lake which experiences drastic seasonal changes in its water level. Lake Poyang comprises a permanent water body and seasonally flooding littoral zone. As shown in Figure 1, the littoral wetland is usually inundated when the water level is relatively high (June to August) and uninundated when the water level is relatively low (September to the next May). During the uninundated period, the littoral zone of Lake Poyang is dominated by Carex species, which has two growing seasons: the autumn growing season, from September to December, and the spring growing season, from February to May. The main vegetation of this site is Carex cinerascens, followed by Eleocharis valleculosa, Cardamine lyrata and Potentilla limprichtii. This littoral wetland is covered with meadow soil on the ground surface, characterized as sandy loam. Table 1 shows the main characteristics of soil in this littoral wetland. Additionally, this study area was
an unexplored nature reserve of Jiangxi Province, China, and there was no water on the ground during the entire experimental period.

![Figure 1](image-url)

**Figure 1.** Variation of (a) surface water level and (b,c) inundation area of Lake Poyang. Panel (a) shows the average monthly water level from 1953 to 2015 at Xingzi station (a representative hydrological station of Lake Poyang). Panel (b,c) are the schematic diagrams of the inundation area of Lake Poyang for (b) the uninundated period and (c) the inundated period.

**Table 1.** Soil characteristics under control and warming (mean ± standard error). TN: total nitrogen; SOC: soil organic carbon.

| Soil Characteristics | Control       | Warming       |
|----------------------|---------------|---------------|
| Soil pH              | 4.9 ± 0.1     | 4.8 ± 0.1     |
| $NH_4^+$ N (mg kg$^{-1}$) | 7.4 ± 0.6     | 9.4 ± 1.2     |
| NO$_3^-$ N (mg kg$^{-1}$) | 6.3 ± 0.9     | 8.8 ± 2.0     |
| TN (g kg$^{-1}$)      | 1.0 ± 0.1     | 1.0 ± 0.1     |
| SOC (g kg$^{-1}$)     | 9.7 ± 1.2     | 9.9 ± 1.1     |

2.2. Experimental Design

The experiment was manipulated as a completely random design with two different treatments: control and warming. Each treatment was replicated in four blocks. In total, eight plots were established with an area of 2 m × 2 m for each plot. The distance between any block was over 5 m and that between each adjacent plot was over 2 m. A passive experimental warming device—open-top chambers (OTCs)—was used to simulate a warming environment following the instructions of the International Tundra Experiment [33,34]. OTCs have been verified effectively and are widely used in multiple terrestrial ecosystems, including forests [35], grasslands [36,37], and wetlands [2,38]. In this experiment, OTCs were made of transparent synthetic glass with a 5 mm thickness. OTCs were 80 cm tall and hexagonal with a top area of 0.6 m$^2$ and a bottom area of 2.6 m$^2$. At the end of August 2018, OTCs were installed and kept in place throughout the whole experimental period.

2.3. Gas Sampling and Analysis

$N_2O$ flux was measured using the opaque static chamber and gas chromatograph technique during the uninundated period from September 2018 to May 2019. The static chamber was made of PVC, including two parts: a top chamber and a base collar. The base collar was equipped with a groove on the top and was buried under ground at a 10 cm depth before the experimental site was inundated. The top chamber was 30 cm in height and 30 cm in diameter. The groove was filled with some water and fitted to the top chamber to generate a closed environment. Then, gas samples were collected from the closed chamber using a 50 mL syringe every 10 min for half an hour.
Measurements were conducted between 9:00 to 15:00 local time, twice a month (except February and April 2019). Gas samples were taken to a laboratory and analyzed within three days after sampling. A gas chromatograph (Agilent 7890B, Agilent Technologies, CA, USA) equipped with an electron capture detector was used to analyze N\textsubscript{2}O concentration. N\textsubscript{2}O flux was calculated with R [39] and the package “flux” [40]. The normalized root-mean-square error (NRMSE) and coefficient of determination (R\textsuperscript{2}) were used to screen the flux data following the procedure of Minke et al. (2016) [41]. In total, 1% of the measurements were therefore rejected.

2.4. Environmental Parameters

A micrometeorological station (HOBO USB Micro Station, Onset Computer Corporation, Bourne, MA, USA) was established at the study site to record precipitation (S-RGB-M002) and air temperature (both control and warming plots, S-TMB-M002) at an interval of 30 min by a data logger (H21-USB). Surface soil (5 cm depth) temperature and moisture in both control and warming plots were recorded automatically every 10 min using a 5TE sensor and Em50 data logger (Decagon Devices, Pullman, WA, USA). In addition, the air temperature of the top chamber, soil temperature, and soil moisture at a 5 cm depth were measured manually for each plot by a thermometer inserted into the top chamber and a portable ProCheck device (Decagon Devices, Pullman, WA, USA), respectively, when sampling gas.

Soil samples were collected from each plot at depths of 0–10 cm in May 2019. In the laboratory, soil samples were air-dried, root-removed, and sieved through a 2 mm mesh for further analysis. Soil pH was measured in ultrapure water at a water to soil ratio of 2.5:1, using a pH meter (PHS-3C, INESA Instrument, Shanghai, China). For the analysis of soil NH\textsubscript{4}\textsuperscript{+}-N and NO\textsubscript{3}\textsuperscript{-}-N, 10 g soil was extracted with 100 mL of 2 mol L\textsuperscript{-1} KCl solution. Then, the solution was shaken for one hour and stood for 30 min. After the soil suspension was filtered, soil NH\textsubscript{4}\textsuperscript{+}-N and NO\textsubscript{3}\textsuperscript{-}-N were analyzed with the Continuous Flow Analyzer (San plus, SKALAR, Breda, The Netherlands). For the analysis of soil organic carbon (SOC), 10 mL of 3 mol L\textsuperscript{-1} HCl was added to an approximately 0.5 g soil sample for 12 h to remove the carbonates. Then, the soil sample was centrifuged and washed with ultrapure water several times until it became neutral. It was freeze-dried and sieved through a 0.075 mm mesh. Approximately 10 mg of soil was taken into a tin cup, and SOC was analyzed with the Elemental Analyzer (EA300, EuroVector, Pavia, Italy). In addition, another 10 mg soil sample was weighed after being sieved directly through a 0.075 mm mesh, and total nitrogen (TN) was also measured by the Elemental Analyzer.

2.5. Data Analysis

A linear mixed-effect model with repeated measurements was applied to test the effect of warming on N\textsubscript{2}O flux using R [39] and the package “lme4” [42]. The model was constructed with the fixed factors of warming by sampling date and the random factors of plot nested within block. In addition, the effects of environmental parameters on N\textsubscript{2}O flux were also analyzed with the model, in which air temperature, soil temperature and soil moisture were taken as the fixed factors and plot nested within block were taken as the random factors. Regression analysis was applied to test the potential dependency of N\textsubscript{2}O flux on air temperature, soil temperature, and soil moisture. All significant differences were considered at the 0.05 level.

3. Results

3.1. Environmental Parameters

Total precipitation was 806.6 mm during the experimental period, with more than 60% of precipitation occurred in the spring growing season (Figure 2a). Warming increased average air temperature by 1.6 °C (Figure 2b).
Soil moisture decreased by 11% and soil temperature increased by 0.4 °C under the warming treatment compared to control (Figure 2c,d). The warming effect of OTCs on air temperature was stronger in the spring growing season than in the autumn growing season; the inverse was true for soil moisture and soil temperature. Soil pH, $NH_4^+$-N, $NO_3^-$-N, TN, and SOC were not significantly affected by warming (Table 1). Despite the statistical significance, warming increased $NH_4^+$-N by 27% and $NO_3^-$-N by 40% compared to the control.

3.2. Effect of Warming on $N_2O$ Flux

Over the entire period, $N_2O$ flux ranged from −1.5 to 21.9 μg m$^{-2}$ h$^{-1}$ under the control treatment and 0.9 to 41.3 μg m$^{-2}$ h$^{-1}$ under the warming treatment (Figure 3). The lowest and highest values of $N_2O$ flux occurred in November 2018 and May 2019, respectively. The average $N_2O$ flux was 8.9 μg m$^{-2}$ h$^{-1}$ under the control treatment, indicating that the littoral zone of Lake Poyang was an $N_2O$ source during the uninundated period.

According to the analysis results of the linear mixed-effects model, we found that warming had a significant effect on $N_2O$ flux, as did the sampling date (Table 2). However, the warming effect did not vary with sampling date. The average $N_2O$ flux was 13.8 μg m$^{-2}$ h$^{-1}$ under warming treatment. Compared with $N_2O$ flux under control treatment, warming significantly increased $N_2O$ flux by 54% (Figure 4). From the different growing seasons, the average $N_2O$ flux under control treatment was 6.2 μg m$^{-2}$ h$^{-1}$ in the autumn growing season and 13.1 μg m$^{-2}$ h$^{-1}$ in the spring growing season, respectively. $N_2O$ flux increased by 19% under the warming treatment in the autumn growing season (7.4 μg m$^{-2}$ h$^{-1}$), but not significantly, while it significantly increased by 85% under the warming treatment in the spring growing season (24.2 μg m$^{-2}$ h$^{-1}$) (Figure 4). In addition, the $N_2O$ flux under both treatments in the spring growing season was significantly higher than in the autumn growing season (Figure 4).
For both treatments, soil moisture had a significant positive correlation with N$_2$O flux between N$_2$O flux and soil moisture, while the relationship between N$_2$O flux and temperature (air temperature and soil temperature) was undetectable (Table 2). For both treatments, soil moisture had a significant positive correlation with N$_2$O flux and explained about 7% of N$_2$O flux variation (Figure 5). There was no significant correlation between N$_2$O flux and soil moisture under control treatment. Moreover, a significant relationship between N$_2$O flux and temperature (air temperature and soil temperature) was undetectable (Table 2).

$$\text{N}_2\text{O flux (μg m}^{-2}\text{h}^{-1}) = 1 + \text{Date} + \text{Warming} \times \text{Date} + \text{Air temperature} + \text{Soil temperature} + \text{Soil moisture}$$

Figure 3. Temporal variation of N$_2$O flux in the uninundated period under the control and warming treatments. The bars indicate standard error.

### Table 2. Statistical results of the linear mixed-effects model for N$_2$O flux.

| Factors                  | Df | p Value |
|--------------------------|----|---------|
| Warming                  | 1  | <0.05   |
| Date                     | 15 | <0.05   |
| Warming × Date           | 15 | 0.21    |
| Air temperature          | 1  | 0.21    |
| Soil temperature         | 1  | 0.83    |
| Soil moisture            | 1  | <0.05   |

Figure 4. Average N$_2$O flux under the control and warming treatments. The uninundated period includes two growing seasons: the autumn growing season and the spring growing season. The bars indicate standard error.

#### 3.3. Dependency of N$_2$O Flux on Soil Temperature and Soil Moisture

A significant correlation was observed between N$_2$O flux and soil moisture, while the relationship between N$_2$O flux and temperature (air temperature and soil temperature) was undetectable (Table 2). For both treatments, soil moisture had a significant positive correlation with N$_2$O flux and explained...
about 7% of N\textsubscript{2}O flux variation (Figure 5). There was no significant correlation between N\textsubscript{2}O flux and soil moisture under control treatment. Moreover, a significant positive correlation was found between N\textsubscript{2}O flux and soil moisture under warming treatment, indicating that warming could increase the relationship between N\textsubscript{2}O flux and soil moisture.

![Figure 5. Relationship between N\textsubscript{2}O flux and soil moisture.](image)

### 4. Discussion

#### 4.1. N\textsubscript{2}O Flux from Different Wetlands

Previous studies have reported a great variability of N\textsubscript{2}O emission or uptake from different wetlands due to various environmental factors [15,30,43–49]. Overall, swamps, peatlands, and marshes in cold regions usually acted as an N\textsubscript{2}O sink or an N\textsubscript{2}O source, while most littoral wetlands emitted N\textsubscript{2}O and showed high variation among different locations (Table 3). On the one hand, subtropical and tropical wetlands usually have warmer weather and store more carbon than cold wetlands [50]. This warm environment is more suitable for microbial activity, and thus more N\textsubscript{2}O is emitted from subtropical and tropical wetlands. On the other hand, littoral wetlands usually experience drastic water level fluctuation and have frequent organic matter transfers between water bodies and lands [26]. These unique thermal conditions and water regimes may determine the greater amount of N\textsubscript{2}O emission from warm littoral wetlands than other wetlands.

#### Table 3. Comparison of N\textsubscript{2}O flux among different wetlands from other studies.

| Wetland Types   | Location                        | N\textsubscript{2}O Flux \(^1\) | Reference |
|-----------------|---------------------------------|-------------------------------|-----------|
| Alpine swamp    | Qinghai-Tibetan Plateau, China  | \(-2.0 \pm 1.2\)              | [15]      |
| Peatland        | Newfoundland, Canada            | \(-23.8 \pm 19.0\)            | [43]      |
| Peatland        | Rzecin wetland, Poland          | \(1.8 \pm 3.7\)               | [44]      |
| Freshwater marsh| Sanjiang Plain, China           | \(4.7^{2} - 1.2^{3}\)        | [45]      |
| Estuarine marsh | Chongming Island, China         | \(-10.6 \pm 2.6 - 32.4 \pm 14.0\) | [46] |
| Littoral wetland| Lake Nakaumi, Japan             | \(-10.0\)                     | [47]      |
| Littoral wetland| Lake Kevätö, Finland            | \(-3.1 \pm 15.7 - 69.1 \pm 15.7\) | [48] |
| Littoral wetland| Lake Huahu, Zoige, China        | \(251.4\)                     | [49]      |
| Littoral wetland| Lake Poyang, China              | \(20.0\)                      | [30]      |
| Littoral wetland| Lake Poyang, China              | \(8.9 \pm 1.1\)               | This study |

\(^1\) The unit of N\textsubscript{2}O flux was \(\mu g\) N\textsubscript{2}O m\(^{-2}\) h\(^{-1}\). Data shown as mean ± standard error. \(^2\) Non-waterlogged marsh. \(^3\) Seasonally waterlogged marsh.

In this study, the littoral zone of Lake Poyang was an N\textsubscript{2}O source during the uninundated period, with a mean flux rate of 8.9 ± 1.1 \(\mu g\) N\textsubscript{2}O m\(^{-2}\) h\(^{-1}\). This result confirms our first hypothesis and is also in line with the result reported by Hu et al. (2016) in the south littoral zone of Lake Poyang [30].
Moreover, N$_2$O flux in the littoral zone of Lake Poyang was much lower than that of Lake Huahu in the Qinghai-Tibetan Plateau [49], which may be attributed to the lower available nitrogen and the non-waterlogged condition in this study site during the sampling period. When considering the inundated period, N$_2$O flux from the water-air interface in the same littoral zone of Lake Poyang was 29.6 ± 10.7 µg N$_2$O m$^{-2}$ h$^{-1}$ (unpublished data), which was more than three times the flux in the uninundated period. Therefore, N$_2$O emission in the littoral zone of Lake Poyang may be greatly influenced by water regimes, meaning that this impact cannot be overlooked when estimating the whole lake’s N$_2$O budget.

4.2. Effect of Warming on N$_2$O Flux

Consistent with previous results in other wetlands [2,15], we found that warming significantly increased N$_2$O flux in this littoral wetland during the uninundated period. This result confirmed our second hypothesis that warming had a positive effect on N$_2$O emission. In the present study, the littoral zone of Lake Poyang varied between being a sink and a source of N$_2$O on an intra-annual timescale. Warming transformed this littoral wetland to a net N$_2$O source during the experimental period. In addition, warming had a much stronger impact on N$_2$O flux in the spring growing season than in the autumn growing season. N$_2$O production is primarily regulated by two microbial activities—nitrification and denitrification—which are highly sensitive to temperature [51]. Warming increased air temperature by 1.6 °C and soil temperature by 0.4 °C, especially during the spring growing season, which can promote both microbial processes. Moreover, N$_2$O flux was significantly related to soil moisture, only accounting for 7% of N$_2$O flux variation, but not to soil temperature. Thus, the third hypothesis was rejected. In other words, N$_2$O flux may be mainly affected by biotic factors (e.g., plants and microbes), rather than abiotic factors (e.g., soil temperature and soil moisture) in the littoral zone of Lake Poyang during the uninundated period.

A recent study found that plant biomass can explain 17–30% of N$_2$O flux variation in the littoral zone of Lake Poyang [30]. In addition, we found that warming increased the content of soil NH$_4^+$-N and soil NO$_3^-$-N, which were the two main substrates supporting the processes of nitrification and denitrification. Therefore, warming may lead to more N$_2$O production than N$_2$O consumption, which further determines N$_2$O emission to some extent. Unfortunately, we are currently unaware of the effects of microbial activities on N$_2$O flux in this littoral wetland. These results suggest that future research should pay more attention to N$_2$O emission pathways and related functional microbes.

4.3. Implication of N$_2$O Emission in the Littoral Wetland under Climate Warming

There are great spatial and temporal variabilities in N$_2$O production, consumption, and emission across different wetlands [52,53], making estimations of N$_2$O budgets uncertain and difficult. Given the impacts of global climate change and anthropic activities, the nitrogen cycle has been drastically disrupted, and this has increased the inventory of N$_2$O [54,55]. As such, it is necessary to investigate N$_2$O flux from various wetlands to fully understand the global N$_2$O budget. Moreover, littoral wetlands have been identified as hotspots of N$_2$O emission according to numerous studies [27,49]. Analogously, the littoral zone of Lake Poyang was an N$_2$O source in the present study, and short-term warming can largely promote N$_2$O emission. Unfortunately, the global temperature rise is likely to exceed 2.0 °C by the end of this century under different Representative Concentration Pathways [1], consequently increasing N$_2$O emission from such littoral wetlands and generating a positive feedback effect to climate warming. Therefore, subtropical littoral wetlands may transform from a relatively weak N$_2$O source to a strong N$_2$O source depending on the level of future climate warming.

Despite not considering two other GHGs—CO$_2$ and CH$_4$—in this study, the contribution of N$_2$O to climate warming should not be ignored due to its large global warming potential. Hu et al. (2016) also concluded that N$_2$O should be integrated into the estimation of net GHG emission for the same lake [30]. In addition, warming effects on GHG fluxes will not always be consistent for a continuous long-term period [18]. Here, we only present a short-term warming experiment, but we do not know
how N$_2$O flux will respond to long-term warming in this littoral wetland. Therefore, the investigation of the key GHGs (CO$_2$, CH$_4$, and N$_2$O) and long-term measurements are required to draw more comprehensive results in such littoral wetlands.

5. Conclusions

This study investigated N$_2$O emission and examined the effect of climate warming on N$_2$O flux in a subtropical littoral wetland. We concluded that the littoral zone of Lake Poyang was an N$_2$O source and that warming significantly increased N$_2$O emission by 54%, indicating that N$_2$O has a positive feedback effect on climate warming. Temperature had no impact on N$_2$O variation, while soil moisture only explained 7% of N$_2$O variation. Moreover, our result indicates that the impacts of the growing season and water regime on N$_2$O should be considered when estimating the whole lake’s N$_2$O budget.

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References

1. IPCC. Climate Change 2013: The Physical Science Basis: Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change; Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M., Eds.; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2013.
2. Laine, A.M.; Mehtätalo, L.; Tolvanen, A.; Frolking, S.; Tuittila, E.-S. Impacts of drainage, restoration and warming on boreal wetland greenhouse gas fluxes. Sci. Total Environ. 2019, 647, 169–181. [CrossRef]
3. Ward, S.E.; Ostle, N.J.; Oakley, S.; Quirk, H.; Henrys, P.A.; Bardgett, R.D. Warming effects on greenhouse gas fluxes in peatlands are modulated by vegetation composition. Ecol. Lett. 2013, 16, 1285–1293. [CrossRef] [PubMed]
4. Schlesinger, W.H. An estimate of the global sink for nitrous oxide in soils. Glob. Chang. Biol. 2013, 19, 2929–2931. [CrossRef]
5. Saunders, D.L.; Kalff, J. Nitrogen retention in wetlands, lakes and rivers. Hydrobiologia 2001, 443, 205–212. [CrossRef]
6. Davidson, E.A.; Janssens, I.A. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 2006, 440, 165–173. [CrossRef] [PubMed]
7. Ravishankara, A.R.; Daniel, J.S.; Portmann, R.W. Nitrous oxide (N$_2$O): The dominant ozone-depleting substance emitted in the 21st century. Science 2009, 326, 123–125. [CrossRef]
8. Fluegge, K. Does environmental exposure to the greenhouse gas, N$_2$O, contribute to etiological factors in neurodevelopmental disorders? A mini-review of the evidence. Environ. Toxicol. Pharmacol. 2016, 47, 6–18. [CrossRef]
9. Massara, T.M.; Malamis, S.; Guisasola, A.; Baeza, J.A.; Noutsopoulos, C.; Katsou, E. A review on nitrous oxide (N$_2$O) emissions during biological nutrient removal from municipal wastewater and sludge reject water. Sci. Total Environ. 2017, 596, 106–123. [CrossRef]
10. WMO. Greenhouse Gas Bulletin: The State of Greenhouse Gases in the Atmosphere Based on Global Observations through 2018; WMO: Geneva, Switzerland, 2019.

11. Lenhart, K.; Behrendt, T.; Greiner, S.; Steinkamp, J.; Well, R.; Giesemann, A.; Keppler, F. Nitrous oxide effluxes from plants as a potentially important source to the atmosphere. New Phytol. 2019, 221, 1398–1408. [CrossRef]

12. Voigt, C.; Lamprecht, R.E.; Marushchak, M.E.; Lind, S.E.; Novakovskiy, A.; Aurela, M.; Martikainen, P.J.; Biasi, C. Warming of subarctic tundra increases emissions of all three important greenhouse gases—Carbon dioxide, methane, and nitrous oxide. Glob. Chang. Biol. 2017, 23, 3121–3138. [CrossRef]

13. Liu, L.; Hu, C.; Yang, P.; Ju, Z.; Olesen, J.E.; Tang, J. Experimental warming-driven soil drying reduced N_2O emissions from fertilized crop rotations of winter wheat–soybean/fallow, 2009–2014. Agric. Ecosyst. Environ. 2016, 219, 71–82. [CrossRef]

14. Zhao, Z.; Dong, S.; Jiang, X.; Liu, S.; Ji, H.; Li, Y.; Han, Y.; Sha, W. Effects of warming and nitrogen deposition on CH_4, CO_2 and N_2O emissions in alpine grassland ecosystems of the Qinghai-Tibetan Plateau. Sci. Total Environ. 2017, 592, 565–572. [CrossRef]

15. Chen, X.; Wang, G.; Zhang, T.; Mao, T.; Wei, D.; Song, C.; Hu, Z.; Huang, K. Effects of warming and nitrogen fertilization on GHG flux in an alpine swamp meadow of a permafrost region. Sci. Total Environ. 2017, 601–602, 1389–1399. [CrossRef] [PubMed]

16. Qiu, Y.; Jiang, Y.; Guo, L.; Burkey, K.O.; Zobel, R.W.; Shew, H.D.; Hu, S. Contrasting warming and ozone effects on denitrifiers dominate soil N_2O emissions. Environ. Sci. Technol. 2018, 52, 10956–10966. [CrossRef] [PubMed]

17. Chen, X.; Wang, G.; Hu, Z.; Song, C.; Liang, Y.; Wang, J.; Song, X.; Lin, S. The effect of nitrogen deposition rather than warming on carbon flux in alpine meadows depends on precipitation variations. Ecol. Eng. 2017, 107, 183–191. [CrossRef]

18. Zhu, X.; Luo, C.; Song, W.; Zhang, Z.; Cui, S.; Bao, X.; Jiang, L.; Li, Y.; Li, X.; Wang, Q.; et al. Effects of warming, grazing/cutting and nitrogen fertilization on greenhouse gas fluxes during growing seasons in an alpine meadow on the Tibetan Plateau. Agric. For. Meteorol. 2015, 214–215, 506–514. [CrossRef]

19. Shi, F.; Chen, H.; Chen, H.; Wu, Y.; Wu, N. The combined effects of warming and drying suppress CO_2 and N_2O emission rates in an alpine meadow of the eastern Tibetan Plateau. Ecol. Res. 2012, 27, 725–733. [CrossRef]

20. Chen, X.; Wang, G.; Zhang, T.; Mao, T.; Wei, D.; Hu, Z.; Song, C. Effects of warming and nitrogen fertilization on CH_4, CO_2 and N_2O emissions in alpine grassland ecosystems of the Qinghai-Tibetan Plateau. Atmos. Environ. 2017, 157, 111–124. [CrossRef]

21. Hu, Y.; Chang, X.; Lin, X.; Wang, Y.; Wang, S.; Duan, J.; Zhang, Z.; Yang, X.; Luo, C.; Xu, G.; et al. Effects of warming and grazing on N_2O fluxes in an alpine meadow ecosystem on the Tibetan plateau. Soil Biol. Biochem. 2010, 42, 944–952. [CrossRef]

22. Yue, Q.; Wu, H.; Sun, J.; Cheng, K.; Smith, P.; Hillier, J.; Xu, X.; Pan, G. Deriving emission factors and estimating direct nitrous oxide emissions for crop cultivation in China. Environ. Sci. Technol. 2019, 53, 10246–10257. [CrossRef]

23. Dijkstra, F.A.; Morgan, J.A.; Follett, R.F.; LeCain, D.R. Climate change reduces the net sink of CH_4 and N_2O in a semiarid grassland. Glob. Chang. Biol. 2013, 19, 1816–1826. [CrossRef] [PubMed]

24. Chen, D.; Fu, X.-Q.; Wang, C.; Liu, X.-L.; Li, H.; Shen, J.-L.; Wang, Y.; Li, Y.; Wu, J.-S. Nitrous oxide emissions from a masson pine forest soil in subtropical central China. Pedosphere 2015, 25, 263–274. [CrossRef]

25. Vogt, J.; Wu, J.; Altdorff, D.; Le, T.B.; Gong, Y. Nitrous oxide fluxes of a boreal abandoned pasture do not significantly differ from an adjacent natural bog despite distinct environmental conditions. Sci. Total Environ. 2020, 714, 136648. [CrossRef] [PubMed]

26. Larmola, T.; Alm, J.; Jauhainen, S.; Martikainen, P.J.; Silvola, J. Ecosystem CO_2 exchange and plant biomass in the littoral zone of a boreal eutrophic lake. Freshw. Biol. 2003, 48, 1295–1310. [CrossRef]

27. Li, S.; Bush, R.T.; Santos, I.R.; Zhang, Q.; Song, K.; Mao, R.; Wen, Z.; Lu, X.X. Large greenhouse gases emissions from China’s lakes and reservoirs. Water Res. 2018, 147, 13–24. [CrossRef]

28. Zhao, X.; Liu, Y. Variability of surface heat fluxes and its driving forces at different time scales over a large ephemeral lake in China. J. Geophys. Res. Atmos. 2018, 123, 4939–4957. [CrossRef]

29. Gu, C.; Mu, X.; Gao, P.; Zhao, G.; Sun, W.; Li, P. Effects of climate change and human activities on runoff and sediment inputs of the largest freshwater lake in China, Poyang Lake. Hydrol. Sci. J. 2017, 62, 2313–2330. [CrossRef]
30. Hu, Q.; Cai, J.; Yao, B.; Wu, Q.; Wang, Y.; Xu, X. Plant-mediated methane and nitrous oxide emissions from a carex meadow in Poyang Lake during drawdown periods. *Plant Soil* **2016**, *400*, 367–380. [CrossRef]

31. Zhang, Q.; Gu, X.; Singh, V.P.; Chen, X. Evaluation of ecological instream flow using multiple ecological indicators with consideration of hydrological alterations. *J. Hydrol.* **2015**, *529*, 711–722. [CrossRef]

32. Liu, S.; Chen, Y.; Liu, J. Methane emissions from the littoral zone of Poyang Lake during drawdown periods. *J. Freshw. Ecol.* **2019**, *34*, 37–48. [CrossRef]

33. Hollister, R.D.; Webber, P.J. Biotic validation of small open-top chambers in a tundra ecosystem. *Glob. Chang. Biol.* **2000**, *6*, 835–842. [CrossRef]

34. Marion, G.M.; Henry, G.H.K.; Freckman, D.W.; Johnstone, J.; Jones, G.; Jones, M.H.; Lévesque, E.; Molau, U.; Mølgaard, P.; Parsons, A.N.; et al. Open-top designs for manipulating field temperature in high-latitude ecosystems. *Glob. Chang. Biol.* **1997**, *3*, 20–32. [CrossRef]

35. Xu, Z.; Wan, C.; Xiong, P.; Tang, Z.; Hu, R.; Cao, G.; Liu, Q. Initial responses of soil CO$_2$ efflux and C, N pools to experimental warming in two contrasting forest ecosystems, Eastern Tibetan Plateau, China. *Plant Soil* **2010**, *336*, 183–195. [CrossRef]

36. Li, N.; Wang, G.; Yang, Y.; Gao, Y.; Liu, G. Plant production, and carbon and nitrogen source pools, are strongly intensified by experimental warming in alpine ecosystems in the Qinghai-Tibet Plateau. *Soil Biol. Biochem.* **2011**, *43*, 942–953. [CrossRef]

37. Zong, N.; Geng, S.; Duan, C.; Shi, P.; Chai, X.; Zhang, X. The effects of warming and nitrogen addition on ecosystem respiration in a Tibetan alpine meadow: The significance of winter warming, *Ecol. Evol.* **2018**, *8*, 10113–10125. [CrossRef] [PubMed]

38. Nielsen, C.S.; Michelsen, A.; Strobel, B.W.; Wulf, K.; Banyasz, I.; Elberling, B. Correlations between substrate availability, dissolved CH$_4$, and CH$_4$ emissions in an arctic wetland subject to warming and plant removal. *J. Geophys. Res. Biogeosci.* **2017**, *122*, 645–660. [CrossRef]

39. R Core Team. *R: A Language and Environment for Statistical Computing*, R version 3.6.1; R Foundation for Statistical Computing: Vienna, Austria, 2019.

40. Jurassinski, G.; Koebusch, F.; Guenther, A.; Beetz, S. Flux Rate Calculation from Dynamic Closed Chamber Measurements. R package version 0.3-0. 2014. Available online: https://CRAN.R-project.org/package=flux (accessed on 15 July 2020).

41. Mitsch, W.J.; Bernal, B.; Nahlik, A.M.; Mander, Ü.; Zhang, L.; Anderson, C.J.; Jørgensen, S.E.; Brix, H. Wetlands, carbon, and climate change. *Landsc. Ecol.* **2013**, *28*, 583–597. [CrossRef]

42. Bates, D.; Mächler, M.; Bolker, B.M.; Walker, S.C. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **2015**, *67*, 1–48. [CrossRef]

43. Gong, Y.; Wu, J.; Vogt, J.; Le, T.B. Warming reduces the increase in N$_2$O emission under nitrogen fertilization in a boreal peatland. *Sci. Total Environ.* **2019**, *664*, 72–78. [CrossRef]

44. Juszczak, R.; Augustin, J. Exchange of the greenhouse gases methane and nitrous oxide between the atmosphere and a temperate peatland in central Europe. *Wetlands* **2013**, *33*, 895–907. [CrossRef]

45. Yu, J.; Liu, J.; Sun, Z.; Sun, W.; Wang, J.; Wang, G.; Chen, X. The fluxes and controlling factors of N$_2$O and CH$_4$ emissions from freshwater marsh in Northeast China. *Sci. China-Earth Sci.* **2010**, *53*, 700–709. [CrossRef]

46. Li, Y.; Wang, D.; Chen, Z.; Hu, H. Comprehensive effects of a sedge plant on CH$_4$ and N$_2$O emissions in an estuarine marsh. *Estuar. Coast. Shelf Sci.* **2018**, *204*, 202–211. [CrossRef]

47. Hirota, M.; Senga, Y.; Seike, Y.; Nohara, S.; Kunii, H. Fluxes of carbon dioxide, methane and nitrous oxide in two contrastive fringing zones of coastal lagoon, Lake Nakaumi, Japan. *J. Geophys. Res.-Atmos.* **2003**, *108*, 104421. [CrossRef]

48. Chen, H.; Wang, M.; Wu, N.; Wang, Y.F.; Zhu, D.; Gao, Y.H.; Peng, C.H. Nitrous oxide fluxes from the littoral zone of a lake on the Qinghai-Tibetan Plateau. *Environ. Monit. Assess.* **2011**, *182*, 545–553. [CrossRef] [PubMed]

49. Duan, P.P.; Song, Y.F.; Li, S.S.; Xiong, Z. Responses of N$_2$O production pathways and related functional microbes to temperature across greenhouse vegetable field soils. *Gefoderm* **2019**, *355*, 113904113904. [CrossRef]
52. Jorgensen, C.J.; Struwe, S.; Elberling, B. Temporal trends in N₂O flux dynamics in a Danish wetland—Effects of plant-mediated gas transport of N₂O and O₂ following changes in water level and soil mineral-N availability. *Glob. Chang. Biol.* **2012**, *18*, 210–222. [CrossRef]

53. Kato, T.; Hirota, M.; Tang, Y.H.; Wada, E. Spatial variability of CH₄ and N₂O fluxes in alpine ecosystems on the Qinghai-Tibetan Plateau. *Atmos. Environ.* **2011**, *45*, 5632–5639. [CrossRef]

54. Canfield, D.E.; Glazer, A.N.; Falkowski, P.G. The evolution and future of earth’s nitrogen cycle. *Science* **2010**, *330*, 192–196. [CrossRef]

55. Gruber, N.; Galloway, J.N. An earth-system perspective of the global nitrogen cycle. *Nature* **2008**, *451*, 293–296. [CrossRef] [PubMed]