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Posted Date: 25 September 2023

doi: 10.20944/preprints202309.1611.v1

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Article

Effects of Lotus (*Nelumbo nucifera* Gaertn.) on the Methane Emission in Littoral Zones of a Subtropical Lake, China

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Abstract: Freshwater lakes represent a potential source of methane (CH₄) to the atmosphere. However, the CH₄ emission contribution to the total emission in the littoral zones of lakes, especially emergent macrophytes (e.g., lotus), are poorly known. Lotus, has been cultivated in almost all provinces in China, is not only an aquatic plant but also a kind of vegetables. Two sampling zones (lotus plant and open water) were established in the lake of the middle reaches of Yangtze River. The CH₄ emission was measured using a floating opaque chamber and gas chromatography between April to December of the years 2021 and 2022. The results indicated that the flux of CH₄ emissions ranged from 0.10 to 59.75 mg m⁻² h⁻¹, with an average value of 5.61 mg m⁻² h⁻¹ in the open water, while ranged from 0.19 to 57.32 mg m⁻² h⁻¹, with an average value of 17.14 mg m⁻² h⁻¹ in the lotus plant zone. The maximal CH₄ emissions occurred in July and August for the open water, which was highly related to the air and water temperature; whereas it happened in September for the lotus plant zone, due to the fresh organic matter inputting to sediments, CH₄ transportation by lotus plant, high soil organic carbon content, and the lower dissolved oxygen concentration. Considering the carbon emissions (both CH₄ and CO₂) and plant productivity, although the greater CH₄ emission occurring in lotus plant zone, it could still represent a potential carbon sink (213 g m⁻² yr⁻¹), compared to the open water.

Keywords: methane emission; littoral lake; lotus; carbon sink; greenhouse gases; Yangtze River

1. Introduction

It is estimated that the global lakes greater than 0.002 km² are up to 117 million, covering a total area of 5.0 million km², and corresponding to 3.7% of Earth's non-glaciated land surface [1].

Although the methane (CH₄) emissions of freshwater (lakes and rivers) are usually less substantial than carbon dioxide (CO₂) fluxes [2], the warming potential of CH₄ is 28 times greater than CO₂ over 100-yr time frames [3]. Globally, the CH₄ emissions of freshwater lakes and rivers are about 122–159 Tg yr⁻¹ and accounting for 20% of CH₄ emission to the atmosphere [3,4] and their contribution is expected to increase in the future climate change scenarios [5]. Because of the spatio-temporal variability of CH₄ emission fluxes from lakes, the recent CH₄ emissions were estimated ranging from 8 to 48 Tg yr⁻¹ from global lakes [6,7]. Therefore, it is necessary to further understand the dynamics of the CH₄ source in lakes under different environmental factors.

Deemer et al. [8] indicated that a variety of biological, morphometric, and physical properties have been used as important predictors of CH₄ emission to the atmosphere from freshwater. These factors could be temperature [9,10], water depth [11,12], ecosystem productivity [9,12,13], and surface area [14,15], and *etc.* However, the links between productivity and CH₄ emission has been shown empirically in various freshwaters [12,16,17]. Hence, in the littoral zone of lakes and rivers, most aquatic plants, such as *Typha*, *Nymphaea*, and *Nelumbo*, have not only high productivity (wet weight of 10 kg m⁻²) [18], but also morphological features adapt to waterlogged habitat [19]. Even the littoral

zone area of freshwater was relatively small, but it could contribute to high CH₄ emission [20,21,22]. Moreover, the contribution of plant-dependent CH₄ emission to the total emission could up to 80%–90% in the freshwater with emergent macrophytes [23,24,25]. Therefore, the studies conducted in the boreal lakes revealed that about 66%–81% of CH₄ was released from the littoral zones, demonstrating that it is need to reinforce the estimates of lakes CH₄ release in the vegetated littoral zone [21,22].

Lotus (*Nelumbo nucifera* Gaertn.), as an important aquatic plant and a kind of vegetable, has been cultivated in China for more than 2000 years, especially around the Yangtze River [26]. Hubei, located in the middle reaches Yangtze River, is one of the provinces of largest area in the lotus cultivation [26]. Hong Lake covering about an area of 350 km², is the largest freshwater lake in Hubei province [27, 28]. The lake was originally connected with the Yangtze River in 1950s when the area of the lake was 760 km², and now it is semi-connected to the Yangtze River because of the construction of sluices around the lake between the years 1955 and 1975 [27]. Although the water surface area of the lake has greatly decreased over these decades [27], there are large lotus aquatic plants living in the littoral zone of the lake, which has become a holy place for human recreation. The lotus aquatic plants could affect the CH₄ release of the freshwater, which should be further investigated in the Hong Lake.

The purpose of this study was to evaluate the effect of lotus aquatic plant on CH₄ emissions in the freshwater lake in subtropical China. Two observation sites (lotus plant and open water) were set up in the Hong Lake. CH₄ emission was measured by using the floating opaque chamber and a gas chromatography method. In addition, the driving factor of CH₄ emission, the vegetation biomass, soil organic carbon concentration, temperature, and dissolved oxygen concentration were measured as well in order to investigate the relationships between environmental factors and CH₄ emissions.

2. Materials and Methods

2.1. Study sites

The research area lied in the Hong Lake Natural Reserve in the middle reach of the Yangtze River, located between 29°40'N and 29°58'N, and between 113°12'E and 113°26'E [28]. The Hong Lake is the seventh largest freshwater lake in China and the largest lake in Hubei Province [27]. The lake has a surface area of 344 km² with open-water area of 308 km², a littoral area of 36 km², and a mean water depth of 1.34 m [28,29]. This region is in the northern fringe of a humid subtropical monsoon climate with the average annual temperature of 15.9–16.6 °C and the average annual precipitation of 1060–1331 mm, amongst about 74% of precipitation occurred from April to October [27–29]. The paddy and fluvo-quic soil are the typical soils there. Additionally, the Hong Lake has become one of the international important wetlands of the Ramsar convention in 2008 and one of the Chinese Wetland Ecosystem Research Stations in 2014 [28].

In the present study, two sites in the Hong Lake were selected to monitor CH₄ emission flux in the open water site (KKs) and the emergent vegetation of lotus (*N. nucifera* Gaertn.) site (NNs). No vegetation was grown in the site KKs. While, the NNs site was covered by the emergent vegetation of *N. nucifera* Gaertn with an area of 60 ha, and sparse areas containing *Zizania latifolia* Griseb Stapf., and invasive plant *Eichhornia crassipes* Mart Solme.

2.2. CH₄ measurements

The floating opaque chambers were used to measure CH₄ emission from the open water and *N. nucifera* Gaertn containers. CH₄ gases were sampled from April to December in 2021, and from April to October in 2022 in the Hong Lake, and gas samples were collected once per month on a clear day between 8:30 and 11:30 a.m (Beijing standard time, GMT + 8 h). The chamber was made by the acrylic organic glass (40 cm height × 35 cm diameter) [28]. Air inside the chambers was circulated with battery-driven fans during the measurement to ensure gas samples to be well-mixed. In addition, the top of the chamber was setup a thermometer sensor, while the open-end of the chamber fitted with a cystosepiment and tyre as floating equipment (Fig. 1a). During the measurement, three chambers at each site were placed upside down at a distance of 50 to 100 cm apart on the water surface. The open-end of the chamber remains approximately 10 cm below the water surface. Generally, gas samples of

the chamber air were pulled into 60 mL polypropylene syringes at 0, 5, 10, and 15 min after enclosure. Subsequently, all gas samples were injected into gas bags (0.1 L) and taken to the laboratory for determination within three days.

The hanging opaque chambers were used to measured plant-dependent CH₄ emission in the NNs site (Fig. 1b). The chamber was made by the acrylic organic glass (30 cm height × 15 cm radius). The stem and leaf of the lotus above the water surface were closed the chamber, and the end of the chamber was sealed by two half rounds of acrylic glass plates. In addition, the center of the acrylic glass plate had a small round hole (~3 mm) for passing through the plant, and the edge of the hole was sealed by the plasticine when the plant included in the chamber. Meanwhile, the bottom edge of the chamber was closed with the transparent tape to guard against leakage. the inner of the chamber was set up also the thermometer sensor and fans, its function similar to the floating opaque chambers. During the sampling, the chambers were hanged on the two bamboo poles (Fig. 1b), gas samples were collected at 0, 10, 20, and 30 min after enclosure using a 60-mL polypropylene syringes attached to a three-way stopcock. The gas samples for the plant-dependent CH₄ emission in the NNs site were collected twice, namely, on August 25th and September 21st in 2022.

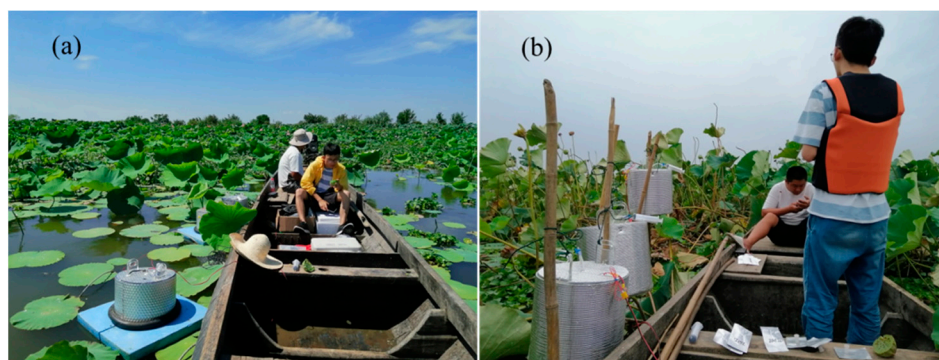


Figure 1. The measuring chamber at the NNs in Hong Lake, including (a) the floating opaque chamber, and (b) the hanging opaque chambers.

The gas samples were determined by gas chromatography (Agilent, 7890A, GC system, Agilent CO., Wilmington, DE, USA) equipped with a flame ionization detector. The gas flux was calculated using the following equation [28]:

$$F = \left(\frac{dc}{dt} \right) \times \left(\frac{M}{V_0} \right) \times \left(\frac{273.15}{T} \right) \times \left(\frac{V}{A} \right) \quad (1)$$

Where F (mg m⁻² h⁻¹) is the CH₄ emission flux at the time of chamber closure; dc/dt (ppm h⁻¹) is the slope of the CH₄ concentration curve variation in the chamber along with time; M and V_0 are the molar mass and molar volume of CH₄ under standard conditions; T (K) is the absolute air temperature in the chamber; V (m³) represents the effective volume of the chamber; A (m²) is the base area of the chamber.

2.3. Determination of the vegetation biomass and environmental factors

The temperatures of air, water temperature, and chamber air, as well as the water depth near the sampling chamber were measured at the same time collecting gas samples. The temperatures of air and inside of chamber were measured using a digital thermometer (TM-902C, Factory of Lihujin Instrument, Guangzhou, China). The water depth at two sites was measured with a ruler and bamboo. Water temperature, pH and dissolved oxygen (DO) concentration at a water depth of 10 cm were measured using a portable multi-parameter water quality meter (Multi 3630 IDS, WTW Co., Munich, Germany).

The vegetation biomass of the NNs site, at an area of 50 cm × 50 cm, were sampled in 2021. Three plant samples were randomly selected at the site, and these samples were over-dried at 70°C for 48 hours, and then weighed. Three soil samples were also collected at a depth of 10 cm, and subsequent the soil samples were transferred to the laboratory and dried at 70°C for 48 hours. The soil samples were milled and passed through a 0.125 mm sieve to determine the soil organic carbon (SOC) content using the potassium dichromate oxidation-external heating method. The soil pH value was measured using the potentiometric method [30]. The total nitrogen (TN) content in soil samples was determined by the Kjeldahl method with H₂SO₄ digestion, and the total phosphorus (TP) content of the soil samples was measured using the coloimetry by alkali fusion with NaOH [28].

2.4. Date analysis

SPSS v 18.0 software was used for data processing and statistical analysis [30]. The Independent-samples *T* test was conducted to analyze the differences in mean CH₄ emission, SOC content, TN, TP, vegetation biomass, and pH in soil. While the Paired *t*-test was conducted to test the differences in the mean of the temperature, pH, DO and water depth. Pearson’s rank correlation coefficient was used to analyze correlations between CH₄ emission and temperatures, water depth, DO, pH. The statistical differences were considered significant at the *P* < 0.05 level.

3. Results

3.1. Environmental Factors

Table 1 showed that the average vegetation biomass at NNs was 798.68 g m⁻². The soil pH at NNs was lower than that at KKs, whereas the SOC content and TN at NNs was two times than that at KKs (*P* < 0.05). There were no significant difference (*P* > 0.05) in the carbon nitrogen ratio (C/N) and TP between the NNs and at KKs (Table 1).

Table 1. The physicochemical characteristics at the Hong Lake.

Sites	Vegetation		Soil				
	Vegetation types	Biomass/g m ⁻²	pH	SOC/g kg ⁻¹	TN /g kg ⁻¹	C/N	TP/g kg ⁻¹
KKs	No the vegetation	—	8.12±0.05a	16.63±1.54a	1.33±0.14a	12.54±0.21a	0.64±0.01a
NNs	<i>N. nucifera</i>	798.68±12.34	7.27±0.08a	35.57±1.67b	3.07±0.18b	11.63±0.40a	0.62±0.02a

Note: different lowercase letters indicate a significant difference exists between two sites.

The mean air temperature was 26.1 and 26.4°C, and the mean water temperature was 24.4 and 24.1°C at KKs and NNs for 2021–2022, respectively. The mean water depth at NNs (108 cm) for the 2021–2022 was significantly (*P* < 0.05) lower than that of the KKs (156 cm). The mean pH at 10 cm water depth in the KKs and NNs was 8.4 and 7.8 for 2021–2022, respectively. In addition, the mean DO concentrations at 10 cm water depth for 2021–2022 at NNs (4.5 mg/L) was significantly (*P* < 0.05) lower than that at KKs (7.9 mg/L) (Fig.2).

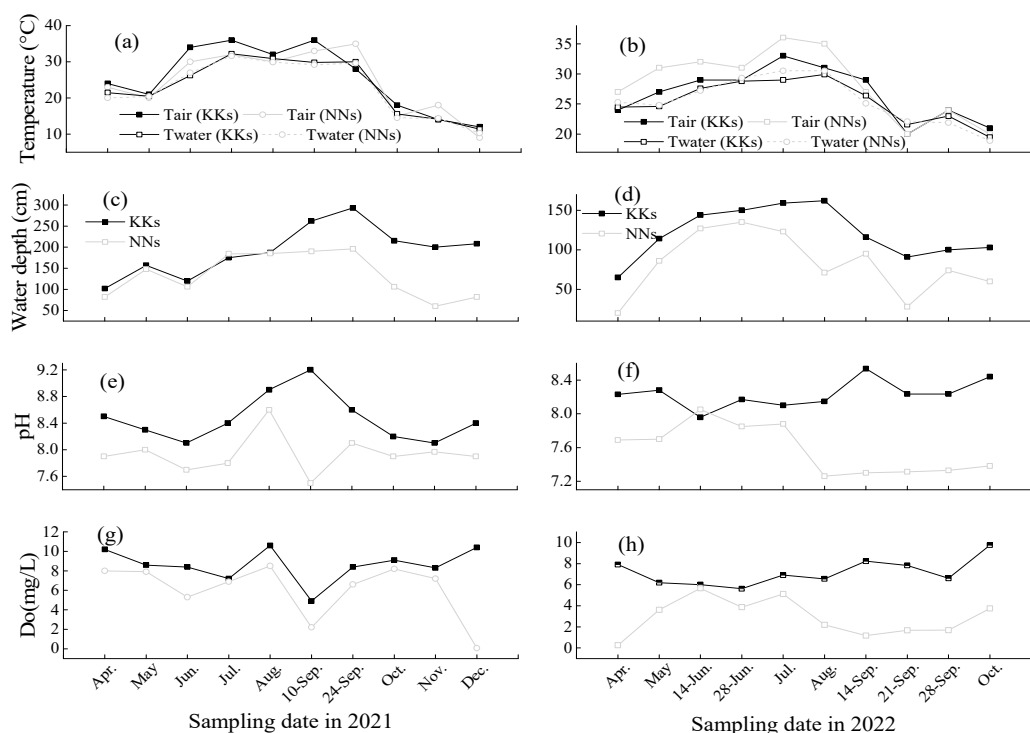


Figure 2. Environmental factors in two sampling sites in the Hong Lake, including (a) air and water temperatures in 2021, (b) air and water temperatures in 2022, (c) water depth in 2021, (d) water depth in 2022, (e) pH in 2021, (f) pH in 2022, (g) dissolved oxygen concentration in 2021 and (h) dissolved oxygen concentration in 2022.

3.2. CH_4 emission flux

The temporal variations in CH_4 emission fluxes for 2021–2022 were recorded at the NNs and KKs, and the peak values of the KKs occurred in July and August, whereas in September at the NNs (Fig.3). The CH_4 emission fluxes ranged from 0.10 to 59.75 $mg\ m^{-2}\ h^{-1}$ at the KKs for 2021–2022, and ranged from 0.19 to 57.32 $mg\ m^{-2}\ h^{-1}$ at the NNs for 2021–2022, respectively (Fig.3).

Plant-mediated CH_4 emission at the NNs was 9.58 and 15.27 $mg\ m^{-2}\ h^{-1}$ on 25th of August and 21st of September, 2022, accounting for 30% and 85% of total emissions, respectively (Fig. 4a). Mean CH_4 emissions from the KKs and NNs were 9.68 and 18.18 $mg\ m^{-2}\ h^{-1}$ for 2021, and were 1.55 and 16.09 $mg\ m^{-2}\ h^{-1}$ for 2022, respectively (Fig. 4b). While the mean CH_4 emission flux ($17.14 \pm 1.98\ mg\ m^{-2}\ h^{-1}$) at the NNs was three times than that at the KKs for 2021–2022 ($5.61 \pm 1.86\ mg\ m^{-2}\ h^{-1}$) (Fig. 4b).

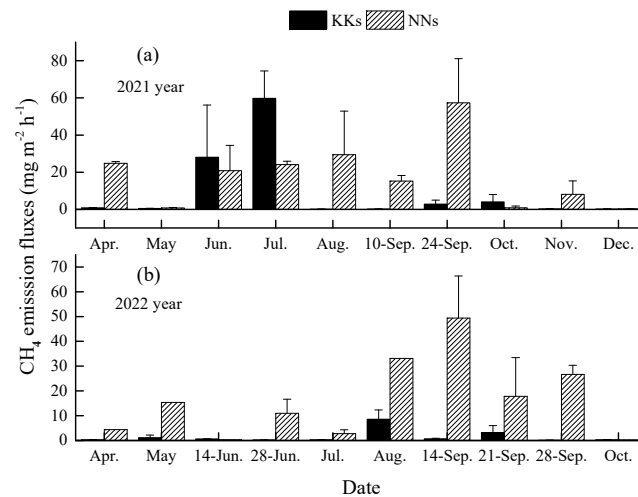


Figure 3. CH₄ emission fluxes in two sites in the Hong Lake, including (a) CH₄ emission in 2021 and (b) CH₄ emission in 2022.

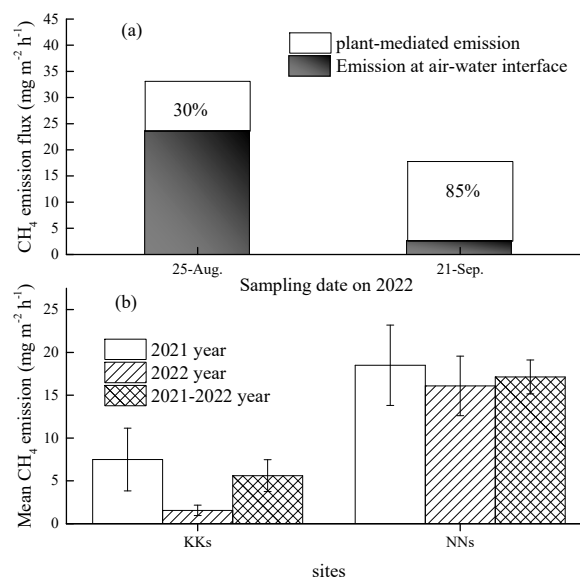


Figure 4. Emission fluxes and plant transport for CH₄ in two sites in the Hong Lake, including (a) the contribution of CH₄ emission transport by plant to total emissions and (b) Mean CH₄ emissions.

3.3. Relationship between CH₄ emission and environmental factors

All CH₄ emission fluxes were significantly ($P < 0.05$) and positively correlated with air and water temperatures (Fig. 5a, b). When two values of the CH₄ emission flux (57.72 and 59.75 mg m⁻² h⁻¹) were removed, the CH₄ emission fluxes were significantly ($P < 0.05$) and negatively correlated with the DO concentration in the water (Fig. 6).

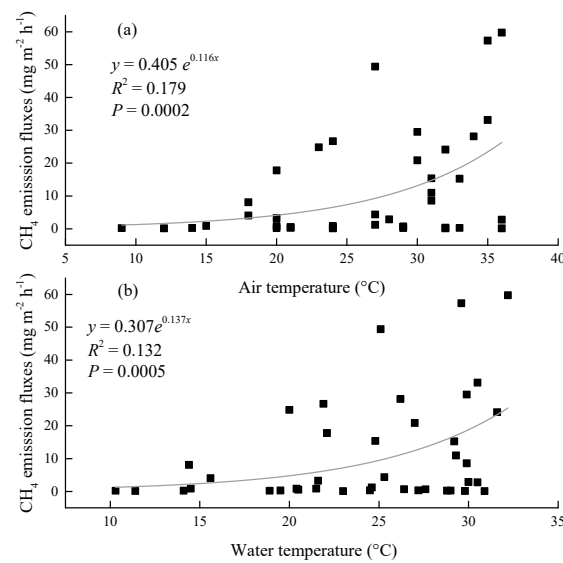


Figure 5. The correlation between CH₄ emission fluxes and temperatures of air and water in Hong Lake.

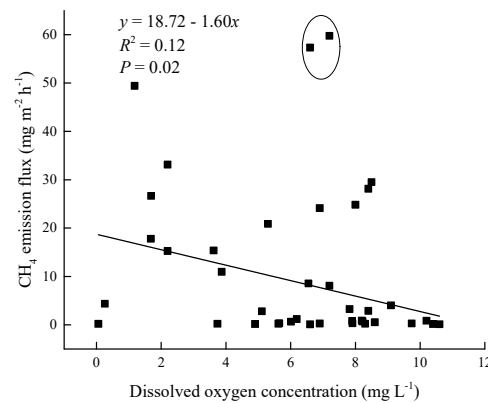


Figure 6. The correlation between CH₄ emission fluxes and DO concentration in Hong Lake.

4. Discussion

In recent years, studies have reported that freshwater lakes are important natural sources of CH₄ to the atmosphere [2,31]. In this study, the CH₄ emission flux shows very large temporal variations at the Hong Lake, with a range from 0.10 to 59.75 mg m⁻² h⁻¹ during 2021–2022 (Fig. 3). The CH₄ emissions in Hong Lake were high and comparable to the Dong Lake of the largest Chinese urban lake (0.06–5.53 mg m⁻² h⁻¹) [32] and the largest shallow eutrophic lake Taihu in Chinese subtropical region (0.006–0.37 mg m⁻² h⁻¹ for the diffusion emission) [33]. However, Wang et al. [34] indicated that the macrophyte-covered littoral zone were the “hotspots” of CH₄ emission in Lake Taihu, ranging from -1.7 to 131 mg m⁻² h⁻¹ from August 2003 to August 2004. In addition, Gondwe et al. [35] also reported that the CH₄ diffusion emission in the swamps in the Okavango Delta, Botswana, varied between 0.24 and 293 mg m⁻² h⁻¹. Hence, the CH₄ emission fluxes in the present study were significantly higher than those results of Xing et al. [32] and Xiao et al. [33], but significantly lower than those results of Wang et al. [34] and Gondwe et al. [35]. The CH₄ emission fluxes measured in

the Hong Lake in this study were within the range reported for other subtropical/ tropical wetlands. The results showed that the CH₄ emission in the lake in different regions was obvious difference.

In our study, the maximal CH₄ emission occurred in the Summer (July and August) and Autumn (September), which were consistent with previous studies [28,34,36]. However, there was significant difference in the temporal variation of CH₄ emission between the KKs and NNs, e.g., maximal CH₄ emissions in July and August for the KKs, while in September for the NNs (Fig. 3). The significant difference of CH₄ emission was ascribed to ecological determinants, e.g., climate (temperature), water depth, and vegetation types. We found that all CH₄ emission fluxes during two growing seasons were significantly and positively related to the air and water temperatures (Fig. 5a, b). The results are in agreement with the observations in previous studies, which revealed that temperature could obviously affect the seasonal CH₄ emission in lakes and peatlands [32,36,38,39]. It is well-established that methanogenic microbial communities of sediments of lake increases exponentially with temperature between 2°C and 30°C [40,41,42]. This could explain the maximum CH₄ emission in July and August in the KKs (Fig. 3).

However, compared with the KKs, the maximum values of CH₄ emission in the NNs occurred in September, which may be linked to the emergent macrophytes, *N. nucifera* Gaertn. On the one hand, numerous studies indicated that vegetation was the key factor of CH₄ release in wetlands and the high emission is attributed to primary production, which could supply organic matter incorporated into the sediment and induce the production of CH₄ by methanogenesis [16,17,21,41]. Table 1 represented that the average vegetation biomass (798.68 g m⁻²) and SOC content (35.57 g kg⁻¹) in the NNs was significantly higher than that of the KKs. And then the high biomass and SOC content should stimulate the production and emission of CH₄, which can lead to the maximal CH₄ emission. However, Kim et al. [39] pointed out that the peak in CH₄ emission flux lagged the peak in biomass production by 2–3 weeks. Burke et al. [43] found the rates of highest CH₄ emission from Florida everglades with the emergent aquatic vegetation, likely attributed to the organic matter incorporated into sediments. On the other hand, numerous literatures provided evidence that the contribution of plant transported through the aerenchyma to total CH₄ emission in the freshwater wetlands was 80%–90% [23,24,25], most of the CH₄ released from the shallow sediment escaped oxidation and reached the atmosphere, it was estimated that about 60%–80% of the CH₄ emission was from the littoral zones in boreal lakes [21,22]. In our studies, the contribution of plant-dependent CH₄ emission to the total emission in the NNs was 30%–85% (Fig. 4a). Hence, the lotus plant zone in Hong Lake has been shown to emit more CH₄ than that of open water (Fig. 3), probably attributed to the supply of fresh organic matter from lotus plant in the littoral zone and greater transport of CH₄ through the plant.

In general, water depth in wetlands is a major factor affecting the spatial and temporal variation of CH₄ emission flux [21,44]. Although we did not found that the significant linear relationship between the CH₄ emission and water depth, there was a significantly negative correlation between the CH₄ emission fluxes and DO concentrations (Fig. 6). One study reported the lower DO concentrations in water overlying the sediment in the lakes led to a higher CH₄ production [45], and thus increased the release of the CH₄ in eutrophic lake [17]. In our study, the average DO concentration (4.5 mg/L) in the NNs was significantly lower than that of the KKs (7.9 mg/L, Fig. 2g, h). Some studies showed that shallow depth zones and near shore areas emitted more CH₄ than other parts of the lakes, which could be attributed not only to rich substrate supply from littoral zone production of organic matter, but also to less time for CH₄ oxidation during passage through water column [14,38,46,47]. This could further explain the higher CH₄ emission fluxes in the NNs, compared to the KKs.

Based on our results, the need for considering the CH₄ emission in the littoral region of freshwater was further emphasized, which could influence the sink of carbon (C) in lakes. Therefore, the average CH₄ emissions (12.86 mg C-CH₄ m⁻² h⁻¹ in the NNs) reported in the present study was higher than the average CO₂ emission (3.78 mg C-CO₂ m⁻² h⁻¹ with no vegetation grew) in Chinese subtropical lakes Donghu reported by Xing et al. [32] in the same climate region. We preliminary estimated that the annual C emission in the NNs was 113 g C m⁻² yr⁻¹ of CH₄ (12.86 mg C-CH₄ m⁻² h⁻¹

$\times 0.001 \times 24 \text{ h} \times 365 \text{ d}$ for the annual estimates of CH_4 emission), and was $33 \text{ g C m}^{-2} \text{ yr}^{-1}$ of CO_2 ($3.78 \text{ mg C-CO}_2 \text{ m}^{-2} \text{ h}^{-1} \times 0.001 \times 24 \times 365 \text{ d}$ for the annual estimates of CO_2 emission), respectively. In addition, the net primary productivity (dry weight) of the emergent vegetation, *N. nucifera* Gaertn was $798.68 \text{ g m}^{-2} \text{ yr}^{-1}$ (Table 1). Subsequently, according to the transformation coefficient of C was usually 0.45, thereby the C fixed by plants was $359.41 \text{ g C m}^{-2} \text{ yr}^{-1}$ ($359.41 \text{ g C m}^{-2} \text{ yr}^{-1}$ minus $113 \text{ g C m}^{-2} \text{ yr}^{-1}$ minus $33 \text{ g C m}^{-2} \text{ yr}^{-1}$ for the budgets of C sink), and thus the C sink for the NNs zone was $213.41 \text{ g m}^{-2} \text{ yr}^{-1}$. Although the greater CH_4 emission in emergent vegetation, *N. nucifera* Gaertn zone, it can still represent a potential sink of carbon.

5. Conclusions

In the present study, the Hong Lake was a potential source of CH_4 to atmosphere. The massive plant biomass, more effective plant-mediated transport of CH_4 , and higher SOC content, could significantly enhanced the release of CH_4 in the emergent vegetation of *N. nucifera* Gaertn. Moreover, the lower DO concentration in water overlying the sediment further stimulated the release of the CH_4 in the emergent vegetation zone. After the combination of the carbon emission (CH_4 and CO_2) and the net primary productivity, the CH_4 emission in emergent vegetation, *N. nucifera* Gaertn zone was larger than that of the open water, but it can still represent a potential sink of carbon.

Author Contributions: Conceptualisation, W.Z., Y.S., L.H., X.X., J.Y., and S.X.; formal analysis, W.Z., and L.H.; funding acquisition, W.Z.; investigation, W.Z., L.H., S.X., X.X., W.O. and T.F.; supervision, S.X., T.F., J.Y., and W.O.; validation, W.Z.; writing—original draft, X.Y., and W.Z.; writing—review and editing, W.Z. and X.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Natural Foundation of China (NO. 31971474).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We thank Yanxia Zuo for their valuable laboratory analyses assistance from the Institute of Hydrobiology, Chinese Academy of Sciences. The authors would like to thank local fishermen and the staff from Administration of Hong Lake National Nature Reserve for their help in collecting data.

Conflicts of Interest: The authors declare no conflict of interest relevant to this study.

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