Association between greenhouse gases and dissolved organic matter composition in the main rivers around Taihu Lake

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ABSTRACT
River ecosystems receive a large amount of organic matter, which will increase the production of greenhouse gases (GHGs), including carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O). Despite extensive research on the distribution of greenhouse gases and dissolved organic matter (DOM), little is known about the associations between greenhouse gases and DOM compositions. In this study, the distributions of GHGs (CO₂, CH₄ and N₂O) and DOM in the overlying water of the main rivers around Taihu Lake were investigated. The results showed that the concentration of GHGs was positively correlated with dissolved organic carbon concentrations. Three-dimensional excitation-emission matrix fluorescence spectroscopy techniques were employed to identify the source of the DOM, which was related to protein-like and humic-like components. The DOM was a combination of terrigenous and endogenous origins. The GHGs (except CO₂) were significantly associated with DOM composition. These results emphasize the importance of the relationship between GHGs (CO₂, CH₄ and N₂O) and DOM compositions in river ecosystems.

Abbreviations: TDS: total dissolved solid; EC: electric conductivity; DO: dissolved oxygen; ORP: oxidation-reduction potential; TN: total nitrogen; TP: total phosphorus; NH₄⁺-N: ammonium; NO₃⁻-N: nitrate; DTC: dissolved total carbon; DOC: dissolved organic carbon; DIC: dissolved inorganic carbon; CDOM: chromophoric dissolved organic matter

1. Introduction
The concentrations of carbon dioxide (CO₂) and methane (CH₄), which are the two major greenhouse gases (GHGs) in the atmosphere, have continued to rise (Stocker et al. 2014), leading to conditions that are a serious threat to human survival and sustainable social
and economic development. The greenhouse effect has become one of the most concerning environmental problems worldwide. As two important GHGs in the atmosphere, methane (CH₄) and carbon dioxide (CO₂) contribute 18% and 64% to the greenhouse effect, respectively. CH₄ and CO₂ in the atmosphere originate from a wide range of sources, and freshwater (rivers, streams, lakes and reservoirs) is an important source of CH₄ and CO₂ in the atmosphere.

River ecosystems are an important link connecting the two carbon pools of the land and the ocean. The carbon input from rivers worldwide into the ocean is about 0.9 Pg/A (Cole et al. 2007), which is an important structural unit of the global carbon cycle that plays a major role in the material cycle of the biosphere, particularly the carbon cycle (Cole et al. 2001). However, studies in the past 30 years have shown that rivers are not only the transport channels for the global carbon cycle, but also play an important role in the transport of terrestrial organic carbon, which participates in the biogeochemical cycle, and directly emits carbon dioxide (CO₂) (Richey 2002; Butman and Raymond 2011; Raymond et al. 2013) and methane (CH₄) (Marie and Marvin 1987; Bastviken et al. 2011; Belger et al. 2011) to the atmosphere through the water gas boundary. Therefore, research on the CO₂ and CH₄ emissions (carbon emissions) of river systems has become a hot topic in ecological research. Now, research on GHGs is focusing on reservoirs (Jiang et al. 2017, Yang et al. 2021), lakes (Yan et al. 2018, Deng et al. 2019), soils (Li et al. 2014) and wetlands (Tian 2018). Huttunen et al. (2003) studied CH₄ emissions from lakes in Finland and reported that the nutritional status of lakes, the oxygen content of the water during winter, and ice cover have an important effect on CH₄ emissions during the following spring. CH₄ produced during ice cover in the winter is released into the atmosphere when the ice melts (3.6–7.9 g CH₄/m²). In addition, the emission pathways (Bastviken et al. 2004) and factors affecting (Gudasz et al. 2010; Walter Anthony et al. 2010; Marotta et al. 2014) GHGs are beginning to be understood. Borges et al. (2018) found agricultural land has an impact on the concentration of GHGs (CO₂, CH₄, and N₂O) in river ecosystems.

Organic matter is the basic raw material of in situ respiration and microbial metabolism in rivers and is also the main carbon source of endogenous CH₄ and CO₂ in rivers. The decomposition of organic matter by microorganisms is an important source of river CO₂. In addition, there is also a lot of CO₂ that is transported laterally from soils and from wetlands (Abril et al. 2014). CH₄ emissions are more closely related to the organic matter in sediments (Baulch et al. 2011; Crawford et al. 2014). Based on an analysis of the global river CH₄ data, a positive correlation has been reported between CH₄ concentrations and dissolved organic carbon (DOC) (Stanley et al. 2016). Organic carbon not only promotes the production of in situ CH₄ as a source of CH₄ production but also consumes O₂ quickly and reduces CH₄ oxidation in bodies of water with high organic matter (Smith et al. 2000; Mayorga et al. 2005). Exogenous input of organic matter significantly stimulates CH₄ production in rivers (Jones et al. 1995). The bioavailability of organic matter and the composition of humus (humin, humic acid and fulvic acid) in rivers may affect the mechanism of river CH₄ production (Garnier et al. 2013). Smemo and Yavitt (2011) reported that some humus stimulates CH₄ production as an electron receptor for CH₄ production. However, Minderlein and Blodau (2010) showed that some humic acids have an inhibitory effect on methanogens, which is not conducive to the production of CH₄ in sediments rich in organic matter. In addition, different aspects of human activities (e.g., population associated with urban areas and croplands) have specific impact on organic matter in aquatic ecosystems. CO₂ and CH₄ increased and O₂ decreased in
streams with increasing fraction of agricultural land cover on the catchment, strongly suggesting enhanced OM degradation in these streams (Lambert et al. 2017).

Chromophoric dissolved organic matter (CDOM) and the distribution of GHGs have attracted extensive attention in recent years. However, associations between them remain largely unknown. The present study determined the distributions of GHGs (CO₂, CH₄ and N₂O) and dissolved organic matter in the overlying water of the main rivers around Taihu Lake, (1) to reveal the distribution of GHGs and water quality parameters; (2) to identify the source of the DOM in the rivers, and (3) to verify the correlations between the GHGs and dissolved organic matter composition.

2. Materials and methods

2.1. Sampling sites

Sampling was conducted in the main rivers around Taihu Lake in December 2021. The details of the sampling sites are given in Figure 1 and Table 1.

2.2. Sample collection

Water samples of 10 cm beneath the air–water interface were collected. A 300 mL aliquot of the water sample was taken in duplicate in 500 mL sealed glass bottles for the determination of CH₄ and N₂O. They were stored with saturated mercuric chloride to inhibit microbial

Figure 1. Distribution of sampling sites in the main rivers around Taihu Lake.

Table 1. Analytical performance parameters for the determination of GHGs.

| Compounds | Retention time (min) | Detection limits |
|-----------|----------------------|------------------|
| CH₄       | 2.786                | 0.20148          |
| CO₂       | 5.339                | 0.36339          |
| N₂O       | 7.081                | 0.02276          |
activity, and used Apiezon® grease to prevent gas exchange. These samples were stored under cool condition and transported immediately to the laboratory for quantification.

2.3. Physical and chemical analytical methods

Water temperature, pH, dissolved oxygen (DO), oxidation-reduction potential (ORP), and electric conductivity (EC) were measured using a water quality testing instrument (YSI 6600, USA). Samples of total nitrogen (TN) analyses were determined by a UV-6100 spectrophotometer (Mapada, Shanghai, China) (Raveh and Avnimelech 1979). Total phosphorus (TP) was analyzed by colorimetry following digestion with K2S2O8 and NaOH (Ebina et al. 1983). Ammonium-nitrogen (NH4+-N) and nitrate-nitrogen (NO3−-N) were measured with AutoAnalyzer 3 (SEAL, Rottenbach, Germany). Dissolved inorganic carbon (DOC) and dissolved inorganic carbon (DIC) were analysed using a HT 1300 N/C analyser (Analytik Jena, Germany).

2.4. Analysis of gas concentration

The dissolved gas concentrations in the samples were measured using the headspace equilibration method (Xiao et al. 2017; Xiao et al. 2019). By injecting ultra-high purity N2 gas (99.999%), a 50 mL aliquot of water was pushed out to form a headspace. Then, shake the glass bottle vigorously for about 10 min to balance the dissolved gases between the residual liquid and the headspace. To determine the values of dissolved gases in the samples, a small gas sample was withdrawn from the equilibrated headspace of the glass bottle through the syringe with three-way valve. The gas samples were measured by a gas chromatograph (Agilent GC7890B, Agilent Technologies Inc., Palo Alto, CA, USA) fitted with a flame ionization detector to detect CO2, N2O and CH4. Validation parameters have been presented in Table 1.

2.5. Statistical analysis

The Statistical Package of the Social Sciences 24.0 software (SPSS 24.0: SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. One-way analysis of variance (ANOVA) and correlation analyses were conducted using bivariate correlation analysis. Relationships between the production of GHGs and the physicochemical parameters were identified by Pearson’s correlation analysis. A P-value < 0.05 was considered significant.

3. Results and discussion

3.1. Physicochemical parameters of water samples

As shown in Table 2, the pH values in the different bodies of fresh water were weakly alkaline (range 7.67–8.32). The pH at site 2 was significantly higher than that at site 6 (P < 0.01). Total dissolved solids (TDS) are the total amount of inorganic minerals dissolved in the water. The TDS concentrations in the samples were 92.73–456.95 mg/L (mean 302.37 mg/L). Water EC indicates the number of ions in the water. The EC values ranged from 88.30 to 407.90 µs/cm (mean 288.85 µs/cm). The DO concentration affects the survival of aquatic organisms and determines the self-purification ability of water. The average DO concentration at the different sites was 13.44 mg/L. The DO concentration at site 3 was significantly higher than that at site 2 (P < 0.05). The mean oxidation-reduction potential (ORP) value in the overlying water was 239.97 mV.
Table 2. Physical and chemical parameters of sampling sites.

| Point | Temperature °C | DO mg/L | EC μs/cm | TDS | pH | ORP mV |
|-------|----------------|---------|----------|-----|----|--------|
| 1     | 8.53 ± 0.21    | 12.81 ± 0.48 | 258.47 ± 3.41 | 245.27 ± 2.63 | 7.86 ± 0.29 | 260.45 ± 21.71 |
| 2     | 5.43 ± 0.84    | 10.13 ± 0.48 | 262.00 ± 5.56 | 271.70 ± 3.38 | 8.32 ± 0.15 | 233.10 ± 9.76  |
| 3     | 4.90 ± 0.46    | 15.63 ± 1.16 | 335.73 ± 4.96 | 352.52 ± 3.70 | 7.97 ± 0.12 | 256.70 ± 12.01 |
| 4     | 5.97 ± 0.81    | 11.36 ± 0.78 | 407.90 ± 56.00 | 456.95 ± 59.29 | 8.03 ± 0.08 | 239.37 ± 13.90 |
| 5     | 4.80 ± 0.62    | 15.16 ± 1.33 | 325.10 ± 30.80 | 343.63 ± 26.91 | 7.96 ± 0.09 | 254.27 ± 3.27  |
| 6     | 5.80 ± 0.69    | 13.37 ± 0.50 | 344.43 ± 29.89 | 353.82 ± 33.66 | 7.67 ± 0.08 | 190.10 ± 30.41 |
| 7     | 4.30 ± 0.07    | 15.62 ± 0.31 | 88.30 ± 6.77  | 92.73 ± 5.53  | 8.21 ± 0.33 | 245.80 ± 23.31 |

Figure 2. Concentrations of TN (a), TP (b), NO₃⁻-N (c) and NH₄⁺-N (d) in the overlying water.

3.2. Contents of nutrients in the water samples

As shown in Figure 2, the TN level varied between 0.46 and 2.07 mg/L (mean 1.19 mg/L). The TN in the sample from point 4 was the highest with an average of 2.07 mg/L. Nitrogen levels varied from 0.15 to 1.10 mg/L for NO₃⁻-N, 0.04 to 0.62 mg/L for NH₄⁺-N, with mean values of 0.46 and 0.26 mg/L, respectively. The NH₄⁺-N level in the sample from point 6 was significantly higher than those in the other samples (P < 0.01). The highest TP level occurred at site 1 (0.10 mg/L), while the lowest value was 0.04 mg/L, which occurred at site 2, with an average of 0.07 mg/L.

3.3. Dissolved organic matter distribution

As shown in Figure 3, DTC concentrations varied from 12.57 to 41.31 mg/L (mean 32.21 mg/L). The average DTC level at site 3 was significantly higher than those (except site 5) in the other samples (P < 0.01). A similar variation was observed at point 3, where the highest DIC concentration occurred, with a mean of 37.65 mg/L. DOC in the samples varied from 0.90 to 5.32 mg/L, and the maximum value occurred at site 5.
3.4. Distribution of GHGs (CO$_2$, CH$_4$ and N$_2$O)

The N$_2$O content in the samples ranged from 0.03 to 0.07 µmol/L (mean 0.04 µmol/L). The highest CH$_4$ level occurred at site 6 (mean 1.21 µmol/L), while the lowest of 0.13 µmol/L occurred at site 1. Significant differences in CH$_4$ were observed among the samples ($P<0.01$) except between sites 1 and 7. The CO$_2$ concentrations at the sampling sites ranged from 34.12 to 59.48 µmol/L (mean 47.48 µmol/L). Similar to the CH$_4$ distribution (Figure 4), the highest CO$_2$ occurred at point 6, and the lowest occurred at point 1.

3.5. The relationships between the physicochemical parameters and gas production

This study analyzed the correlations between the GHGs and the physicochemical parameters. As shown in Table 2, the concentration of N$_2$O was positively related to the NH$_4^+$-N concentration ($P<0.01$). A positive correlation was detected between CO$_2$ production and the NH$_4^+$-N concentration ($P<0.05$). The DOC value was positively correlated with N$_2$O and CO$_2$ ($P<0.05$). In addition, close correlations were observed among the greenhouse gases in this investigation (Table 3). The CO$_2$ concentration was closely correlated with N$_2$O and CH$_4$.

4. Discussion

4.1. CDOM characteristics in the Rivers

The three-dimensional excitation-emission matrix fluorescence (3D EEM) technique was useful to distinguish the origin of the DOM in the samples. The peaks in the fluorescent EEMs of the DOM in the samples had many common characteristics with previously identified peaks (Table 4). To be specific, four main peaks were identified from the
Table 3. Pearson's correlation between the water quality indices and gas production.

| Index  | TN    | TP    | NH₄⁺-N | NO₃⁻-N | DTC   | DIC   | DOC   | N₂O   | CH₄   | CO₂   | T     | DO    | EC    | TDS   | pH    | ORP   |
|--------|-------|-------|--------|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| TN     | 1     |       |        |        |       |       |       |       |       |       |       |       |       |       |       |       |
| TP     | 0.002 | 1     |        |        |       |       |       |       |       |       |       |       |       |       |       |       |
| NH₄⁺-N| -0.288| 0.110 | 1      |        |       |       |       |       |       |       |       |       |       |       |       |       |
| NO₃⁻-N| -0.039| 0.280 | -0.073 | 1      |       |       |       |       |       |       |       |       |       |       |       |       |
| DTC    | -0.089| 0.212 | 0.685**| -0.285 | 1    |       |       |       |       |       |       |       |       |       |       |       |
| DIC    | -0.087| 0.157 | 0.644**| -0.311*| 0.976**| 1    |       |       |       |       |       |       |       |       |       |       |
| DOC    | -0.063| 0.317 | 0.533**| -0.083| 0.672**| 0.494**| 1    |       |       |       |       |       |       |       |       |       |
| N₂O    | 0.102 | 0.039 | 0.609**| 0.286  | 0.174 | 0.091 | 0.387*| 1    |       |       |       |       |       |       |       |       |
| CH₄    | 0.325 | -0.395*| 0.124 | -0.245 | -0.092 | -0.053 | -0.188 | -0.064 | 1    |       |       |       |       |       |       |       |       |
| CO₂    | -0.022| -0.079| 0.424* | 0.125  | 0.217 | 0.146 | 0.364* | 0.367**| 0.384**| 1    |       |       |       |       |       |       |       |
| T      | -0.322| 0.363 | -0.331 | 0.455* | -0.434*| -0.395 | -0.327 | -0.402 | -0.282 | -0.174 | 1    |       |       |       |       |       |       |
| DO     | 0.326 | 0.127 | 0.139 | -0.072 | 0.068 | 0.027 | 0.146 | 0.263 | -0.011 | -0.416 | -0.467**| 1    |       |       |       |       |       |
| EC     | -0.852**| -0.441 | -0.232 | -0.470*| -0.145 | -0.174 | 0.006 | -0.523**| 0.321 | 0.105 | 0.079 | -0.199 | 1    |       |       |       |       |
| TDS    | -0.831**| -0.486*| -0.191 | -0.511*| -0.105 | -0.135 | 0.026 | -0.487**| 0.351 | 0.119 | -0.014 | 0.156 | 0.995**| 1    |       |       |       |
| pH     | 0.290 | 0.212 | 0.181 | 0.282 | 0.215 | 0.257 | 0.006 | 0.204 | -0.245 | -0.175 | -0.324 | 0.150 | -0.308 | -0.287 | 1    |       |       |
| ORP    | -0.019| -0.025| 0.119 | 0.089 | -0.111| -0.120 | -0.034 | 0.067 | 0.001 | -0.550*| 0.294 | 0.297 | -0.101 | -0.123 | -0.448*| 1    |       |
fluorescent EEMs (Figure 5). Component 1 (C1) was observed at the excitation/emission wavelengths (Ex/Em) of 295/344 nm, which have been reported as tryptophan-like substances (designated peak T2). This component is associated with the newly generated DOM (Coble et al. 2014). Component 2 (C2) has been reported as a protein-like substances, which was observed at the Ex/Em of 285/318 nm (peak B) (Table 4). Component 3 (C3) has been identified in a variety of aquatic environments, which is related to microbial humic-like substances (peak M) (Coble et al. 2014) and is considered to represent both autochthonous and allochthonous DOM. Finally, component 4 was located at Ex/Em of 365 (305)/456 nm (designated peak C), which in previous studies was identified to be fluorescence of visible fulvic-like substances (Zhang et al. 2009). Peak C reflects the fluorescence peak formed by exogenous input of humic acid and fulvic acid, mainly from river input and soil leaching, and reflects the characteristics of the land source (Song et al. 2010). Protein-like peaks are components that reflect the fluorescence peaks formed by biodegradable sources, which are generated by the action of microorganisms and phytoplankton (Coble 1996).

Table 4. Major fluorescence peaks and their spectral positions of dissolved organic matter in previous studies (Coble et al. 1998; Coble et al. 2014; Xi et al. 2018).

| Component | Exmax/Emmax (nm) | Fluorescence peak | Description               |
|-----------|------------------|-------------------|---------------------------|
| C1        | 295/344          | T2                | Tryptophan, Protein-like  |
| C2        | 285/318          | B                 | Tyrosine, Protein-like    |
| C3        | 315/397          | M                 | Microbial humic-like      |
| C4        | 365(305)/456     | C                 | Visible fulvic-like       |

Figure 5. Typical EEMs of DOM in the samples.
3D EEM fluorescence spectroscopy is an effective method to identify the source of organic matter in water and soil. The fluorescence index (FI) is the ratio of emission intensity wavelength at 470 nm to the emission intensity at 520 nm, following an Ex of 370 nm, and has been used as a parameter to distinguish CDOM sources (Jiang et al. 2017). The FI value of organic matter from the autogenous source is low (around 1.3), while the FI value of terrestrial organic matter is high (around 1.9) (McKnight et al. 2001). The FI in the samples ranged from 1.35 to 1.77 (mean 1.45), indicating a combination of terrigenous and endogenous sources. The SUVA254 index is used to describe the degree of humification, indicating the composition of aromatic substances in the DOM. The SUVA254 values ranged from 1.14 to 2.13 (Figure 6), and the highest value occurred at site 1, indicating high humification. Edzwald and Tobiason (1999) reported that small molecular proteins are the main DOM fraction present in freshwater with a SUVA254 value < 2, which was consistent with the DOM characterization results.

### 4.2. Associations between DOM composition and greenhouse gases

In the present study, the DOC concentration was positively correlated with the production of N\textsubscript{2}O and CO\textsubscript{2} (Table 3), indicating that the change was related to organic matter mineralization. Many studies have shown that the organic matter is crucial to the distribution of GHGs (Sierra et al. 2020).

The CO\textsubscript{2} emissions occurring at the water–air interface are the most important in rivers. About 77% of the carbon transported from the land into rivers is in the form of CO\textsubscript{2}, and > 90% of it enters the atmosphere through the water–air interface (Johnson 2008). Terrigenous POC, DOC and DIC flow into rivers in runoff, most of which are directly...
discharged in the form of CO₂, while organic carbon and a small amount of CO₂ flow into downstream rivers. Therefore, some studies have shown that CO₂ in the water of river headwaters is mainly derived from terrigenous soil respiration, while CO₂ downstream is mainly derived from internal organic carbon decomposition (Johnson 2008; Mulholland et al. 2001). Endogenous CO₂ is mainly produced by microbial decomposition of organic carbon in freshwater and sediments.

CH₄ is the final product in the mineralization of anaerobic organic matter (Duc et al. 2010). The production of CH₄ requires the use of three types of microorganisms (hydrolytic bacteria, hydrogen-acetic acid-producing bacteria, and methane-producing bacteria) (Lay et al. 1996). In the present study, the CH₄ levels at sites 2 and 6 were higher than those at the other sites. Sampling site 2 was located in a cyanobacterial accumulating area, and sampling site 6 was near the town. Cyanobacterial blooms release lots of organic matter into freshwaters, which is mineralized by microorganisms. In addition, cyanobacterial residues in the sediments are decomposed by microbiota, releasing CO₂ and CH₄. Previous studies have suggested that sediments are important sites for CH₄ production. However, Hu et al. (2017) reported that CH₄ originates from the sediments but is also transformed by organic matter released during the decomposition of the cyanobacteria in water. In the present study, visible fulvic-like FDOM may have had an effect on the CH₄ production of samples (Table 5). The production of CH₄ is facilitated by the accumulation of chromophoric terrestrial material, as observed in lakes (Zhou et al. 2018). In a eutrophic lake, terrestrial CDOM input increases the concentration of CH₄ rather than protein material. Moreover, the high content of terrigenous organic matter contributes to the increase of methane production (Araujo et al. 2018). This could explain the positive relationship between CH₄ and C₄ compounds.

N₂O is produced during the denitrification process (Seitzinger 1988), which is affected by the concentration of NO₃⁻-N. Denitrifiers utilize N₂O as an electron acceptor under hypoxic and low nitrate conditions (Usui et al. 2001). Studies have shown that N₂O is consumed when the NO₃⁻-N concentration in water is < 0.5 mg/L (Johansson et al. 2003). In the present study, N₂O production was significantly related to the NH₄⁺-N concentration (P < 0.01) (Table 3), which was also reported by Yan et al. (2018). Amaral et al. (2021) showed that tryptophan-like matter enhances the production of N₂O rather than the humic-like fraction from microbial or terrestrial sources. However, no significant correlation was found between N₂O and C₄ (Table 5). This may be related to the sampling site. The sources of organic matter in the investigation are complex and there are many influencing factors.

It is necessary to further explore the relationship between GHGs (CO₂, CH₄ and N₂O) and the composition of DOM, and establish the relationship between the impact of carbon emissions on climate change.

5. Conclusions

This study comprehensively investigated the concentrations and characteristics of GHGs, DOM, and water parameters in the main rivers around Taihu Lake. The GHGs were significantly associated with DOM composition. Protein-like and humic-like components were identified from the DOM using the EEM-PARAFAC method. River ecosystems are subjected to increasing environmental perturbations, which affect organic matter composition and hence affect GHG concentrations. It is important to account for the composition of DOM when studying the distribution of GHGs in a river ecosystem.
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Author contributions

Data curation, Fei He; Formal analysis, Jie Ma; Investigation, Jie Ma, Qiuying Lai and Dongyan Pei; Writing – original draft, Fei He; Writing – review & editing, Weixin Li.

Disclosure Statement

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