Variability of benthic methane-derived carbon along seasonal, biological, and sedimentary gradients in a polymictic lake

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Abstract

The spatial and seasonal variations of methane-derived carbon contributions to Chironomus plumosus larvae and oligochaetes biomass at four stations in a polymictic, eutrophic lake were examined to determine which factors (CH4 and/or dissolved oxygen) affect the methane-derived carbon contributions, and quantify impacts of benthic methane-derived carbon on the lake food web. Methane-derived carbon contributions were estimated by a two-source mixing model using δ13C values of monthly particulate organic matter (−29.5 to −20.8‰) and methane-oxidizing bacteria (−85.7‰). Large spatial and seasonal variability of methane-derived carbon contributions to C. plumosus and oligochaetes within the lake (0–38% and 0–32%, respectively) were observed, where contributions varied spatially along a gradient of sediment CH4 concentration, but not dissolved oxygen. The results suggest that for both C. plumosus and oligochaetes, spatial variabilities of the methane-derived carbon contributions are regulated by CH4. Seasonal variability of methane-derived carbon contributions to C. plumosus revealed increases in summer and autumn following increases in CH4. Methane-derived biomass (methane-derived carbon% × biomass) of C. plumosus were also higher in summer and autumn, suggesting transfer of methane-derived carbon to higher trophic organisms may be larger during those seasons, and limited in winter and spring. Although oligochaetes did not show seasonal variability of methane-derived carbon contributions, they provided a consistent source of methane-derived biomass to higher trophic levels throughout the year. Our study demonstrates that two major macroinvertebrates complementarily contribute to fueling methane-derived carbon cycling through trophic transfer in a polymictic lake.

Freshwater lakes and wetland sediments are major sources of methane production and emission (Bartlett and Harriss 1993; Conrad 2009). Methane flux from lakes are estimated to be 8–48 Tg CH4 yr−1, accounting for 6–16% of natural sources, suggesting that lakes are important sources of methane in global methane budgets (Bastviken et al. 2004). Methane is oxidized by methane oxidizing bacteria typically at oxic-anoxic boundaries near the bottom sediment, which is an important process to reduce methane emissions from lakes to the atmosphere (Bastviken et al. 2002). Through ingestion of proliferated methane oxidizing bacteria in the sediment, methane-derived carbon can also contribute to freshwater food webs (Jones and Grey 2011; Grey 2016).

δ13C of biogenic methane in freshwater lakes is as low as −110 to −50‰ dependent upon formation pathway (Whiticar 1999; Deines and Grey 2006; Taipale et al. 2007). During the use of methane by methane oxidizing bacteria, isotopic fractionation leads to further 13C-depletion (3–39‰; Summons et al. 1994; Templeton et al. 2006), whereas, mean trophic enrichment factor between consumers and food sources (Δδ13C) was +0.8‰ (DeNiro and Epstein 1978; Vander Zanden and Rasmussen 2001). Since extremely low δ13C found in benthic macroinvertebrates such as chironomid larvae and oligochaetes cannot be explained by δ13C of particulate and/or sediment organic matter, the low δ13C values can be used as a proxy for transfer of methane-derived carbon to the benthic community. Furthermore, it has been reported that methane-derived carbon can account for up to 70% of chironomid larval biomass (Jones et al. 2008).

Chironomid larvae, which have been intensively studied, are considered to be a major benthic macroinvertebrate to
assimilate methane-derived carbon (Grey 2016). Oligochaetes are also important benthic macroinvertebrates compared to chironomid larvae in aquatic environments, and their biomass is equivalent to or occasionally larger than that of chironomid larvae (e.g., Hirabayashi et al. 1995). Moreover, they are ubiquitously distributed in freshwater habitats even under conditions in which there are no populations of any chironomidae larvae (Ohtaka et al. 2010). However, there have been few studies of oligochaetes methane-derived carbon contribution based on their δ13C variations in aquatic environments, especially limited to arctic lakes (Hershey et al. 2006). Since oligochaetes exhibit substantial hypoxia tolerance similar to chironomid larvae, they must assimilate methane-derived carbon for survival. Moreover, although chironomid larval biomass transition from aquatic environments to terrestrial ecosystems upon adult emergence, oligochaete biomass remain in aquatic environments throughout their life-cycle. Therefore, these two benthic macroinvertebrates should play different roles in methane-derived carbon cycling in aquatic ecosystems.

Dissolved oxygen (DO) concentration in bottom waters and CH4 concentration in sediments are considered to be the main factors determining methane-derived carbon transfer to benthic macroinvertebrates (e.g., Jones et al. 2008; Yasuno et al. 2012; Grey 2016). In stratifying lakes, when DO concentration in the hypolimnion decrease and become hypoxic, a maximum layer of CH4 concentration in the sediment comes to the surface of the sediment. After the collapse of stratification, DO supply to the bottom layer causes methane oxidizing bacteria proliferation, leading to decrease of δ13C of chironomid larvae and methane-derived carbon transfer to the benthos. The threshold DO concentration in the bottom waters was reported to be 2–4 mg L−1 (Jones et al. 2008).

Although there are limited numbers of studies on methane-derived carbon and food webs in shallow, polymeric lakes (Grey et al. 2004; Kelly et al. 2004; Deines et al. 2007; Yasuno et al. 2012; Agasild et al. 2014, 2018; Cremona et al. 2014), a significantly positive relationship was reported between seasonal variations of CH4 concentration in the sediment and δ13C values of chironomid larvae in a shallow eutrophic Lake Izunuma, Japan (Yasuno et al. 2012). The results suggest that methane-oxidizing bacteria production and chironomid reliance on methane-derived carbon are primarily dependent on the CH4 supply (Yasuno et al. 2012). Another study conducted in Lake Võrtsjärv, Estonia reported that the methane-derived carbon contribution to Chironomus plumosus in a dense-macrophyte vegetation zone was higher than a plankton-dominated zone since macrophytes led to lower DO in the bottom water by restricting water circulation (Agasild et al. 2018). The results suggest fluctuating importance of CH4 and DO in regulating methane-derived carbon transfer in polymeric lakes. In order to explore this hypothesis, variations of methane-derived carbon contributions to benthic macroinvertebrates along gradients of sedimentary characteristics (spatial) and seasons (temporal) were investigated, since CH4 and DO concentrations fluctuate along the gradients of these factors (Fig. 1). For example, higher silt composition and organic matter can lead to higher CH4 concentration and lower DO concentration (e.g., Fedorov et al. 2019). As sediment grain size increases (sediment dominant composition changes from silt to sand) and organic matter decrease, there can be lower CH4 concentration and higher DO concentration. Likewise, there can be higher CH4 and lower DO concentrations in late summer and early autumn, and lower CH4 and higher DO concentrations in winter (e.g., Nakamura et al. 1999; Duc et al. 2010).

Based on these conditions, variations of methane-derived carbon contributions to benthic macroinvertebrates can be categorized into four cases along gradients of sedimentary characteristics or seasons: CH4-dependent contribution (case 1), DO-dependent contribution (case 2), DO and CH4 both (case 3), and ecologically dependent contribution, such as habitat preference and feeding behavior of benthic macroinvertebrates (case 4). In cases 1–3, methane-derived carbon contributions show maxima under certain conditions, affected by CH4 and/or DO concentrations along gradients of sedimentary characteristics or seasons, whereas no clear maximum of the methane-derived carbon contribution is found in case 4. For example, the variability of methane-derived carbon contribution observed in Lake Izunuma (Yasuno et al. 2012) can be

![Fig. 1. Illustration of hypothetical methane-derived carbon contribution to benthic macroinvertebrates coupling CH4 concentration and dissolved oxygen (DO) concentration in/on substrate along gradients of sediment grain size (silt-sand) or seasons; case 1 [①]—CH4 dependent methane-derived carbon contribution, case 2 [②]—DO dependent methane-derived carbon contribution, case 3 [③]—DO and CH4 both, and case 4 [④]—ecologically dependent contribution, such as habitat preference of benthic macroinvertebrates.](image)
categorized into case 1 (CH$_4$-dependent). In case 4 (ecologically dependent), when the benthic macroinvertebrates prefer and distribute around redox boundary in the sediment, they opportunistically ingest methane oxidizing bacteria-derived carbon and there can be no clear spatial and temporal variations in methane-derived carbon contributions. In such a case, biological characteristics such as habitat preference and feeding behavior can be an important factor to determine methane-derived carbon contributions in addition to CH$_4$ and DO. To examine the four cases described above, spatial investigations should be conducted within a lake which has different sediment types, but similarity in the overlying water qualities throughout a year. The knowledge of variability of methane-derived carbon contribution along seasonal, biological, and sedimentary gradients can lead to further understanding and prediction of large-scale methane-derived carbon dynamics in freshwater ecosystems.

Previous studies mainly focused on methane-derived carbon contributions (%) to benthic macroinvertebrates (Jones and Grey 2011). To assess quantitatively the methane-derived carbon impact on aquatic food webs, biomass of each benthic macroinvertebrate (g m$^{-2}$) must be considered in addition to methane-derived carbon contributions (%). For instance, although relatively higher methane-derived carbon contributions (%) to chironomid larval carbon biomass were observed in summer and autumn in Lake Izunuma (Yasuno et al. 2012), low abundance of chironomid larvae led to lower ecological impact of methane-derived carbon on the lake food web. To discuss the transfer of methane-derived carbon to higher trophic levels from the benthos, seasonal variations of methane-derived biomass of benthic macroinvertebrates estimated by methane-derived carbon contribution (%) and benthos biomass (g m$^{-2}$) should be examined and related to the feeding behavior of fish during various growth stages and seasons (Kiso and Kumagai 1989).

The objectives of the present study were to clarify the spatial and seasonal variations of methane-derived carbon quantitative contributions to chironomid larvae and oligochaetes in a shallow, eutrophic lake (polymictic), and identify which methane-derived carbon variation patterns (case 1–4, Fig. 1) were dominant. Furthermore, measuring biomass of larval chironomus and oligochaetes simultaneously, we discuss the seasonal variations of methane-derived carbon impact on lake food web based on the benthos.

**Materials and methods**

**Lake Kasumigaura**

Lake Kasumigaura, the second largest lake in Japan, is located in the eastern part of the Kanto Plain, 50 km northeast of Tokyo (Fig. 2). About 1 million people live in the lake’s watershed (1577 km$^2$). Land use in the watershed is 30% forest, 25% paddy field, 25% plowed field, 10% residential, and 10% others. The lake basin is smooth and shallow, with a surface area of 171 km$^2$, a mean depth of 4.0 m, and a maximum depth of 7.3 m. Due to the extremely high loads of organic matter and nutrients, this lake is well known for eutrophication, with mean concentrations of chlorophyll $a$, phosphorus, and nitrogen of 65 $\mu$g L$^{-1}$, 95 $\mu$g L$^{-1}$, and 1.15 mg L$^{-1}$, respectively, measured at the center of the lake from August 1992 to March 1993 (Imai et al. 2001, 2003). Water content (%), ignition loss (%), and total organic carbon (TOC; mg g-dry$^{-1}$) data in Table 1 were reported by the Kanto Regional Development Bureau of the Ministry of Land, Infrastructure, Transport and Tourism, Japan (http://www.ktr.mlit.go.jp/kasumi/kasumi00146.html, accessed on 01 February 2019). In the present study, station names were selected in accordance to the long-term monitoring project in Lake Kasumigaura to compare with over 40 yr of data.

Sediments in the lake are composed mainly of silt in upstream (Sta. 3; 36°07′30.2′′N, 140°22′65.2′′E) and lake center (Sta. 9; 36°02′14.2′′N, 140°24′22.2′′E), and mainly of sand in downstream (Sta. 12; 35°58′59.3′′N, 140°28′33.2′′E) showing lower water content and TOC (Table 1; Fig. 2). Around Sta. 7 (36°03′90.2′′N, 140°13′93.9′′E), since the sediment has been extensively dredged from 1975 to 2009 (http://www.ktr.mlit.go.jp/kasumi/kasumi00026.html, accessed on 26 June 2019), the sediment at Sta. 7 was composed of a mixture of silt and sand, and water content and TOC of the sediment were lower than those of Sta. 3 and 9. Since within-lake variations were much larger in sediment grain size relative to the surface-water quality (Table 1), this lake was appropriate for examining the spatial variations of methane-derived carbon contributions along a gradient of sediment grain size.

**Sampling**

Monthly sampling was conducted at four stations of Lake Kasumigaura (Sta. 3, 7, 9, and 12), described above, from April 2017 to April 2018 aboard the R/V NIES’94 of the National
Institute for Environmental Studies (Fig. 2). Water temperature and DO concentration were measured using a Hydrolab DS5 (OTT Messtechnik GmbH & Co.) at 0.1 m intervals throughout the water column. Transparency was measured by a Secchi disk. Surface-water samples were collected using a 2-m vertical column sampler for nutrients and particulate organic matter (POM). The water samples were immediately cooled in an ice cooler and brought back to the laboratory.

Benthic macroinvertebrates and surface sediment samples were collected using an Ekman grab. Benthic macroinvertebrates were then sieved out from the surrounding sediment (NGG40, mesh size: 475 μm), and then stored in a polyethylene bag. The Ekman grab collection for benthic macroinvertebrates for stable isotope analysis was repeated 5–15 times at each station depending on the abundance of benthic macroinvertebrates. The sampling by the Ekman grab collection for measuring the biomass of benthic macroinvertebrates was repeated three times at Sta. 7 and four times at Sta. 3, 9, and 12. Surface sediment samples for stable isotope analysis were collected by a 10 mL micropipette from the Ekman grab and then put in 50 mL centrifuge tubes. The samples were brought back to the laboratory in a cooler box.

For grain size analysis, sediment samples were collected in July 2019 at each station, and the samples were brought back to the laboratory in a polyethylene bag. For analysis of CH₄ concentration in the surface sediment, the surface sediments (~ 5 cm) were sampled by a 10 mL micropipette from the Ekman grab sampler from January to November 2019. The sediment was placed in a single glass vial, filled with Milli-Q water, and sealed with a butyl rubber cap (sediment vial). Two milliliters of headspace was created using ambient air, and the vial was shaken vigorously for 2 min on-board. After shaking, 1 mL of the air in the headspace from the sediment vial was placed into a second vial already filled with Milli-Q water (methane vial). The methane vial was kept upside-down until analysis. The CH₄ sampling was concluded within 10 min after the sediment sampling. The sediment vial was later used for measuring wet weight of the sediment.

### Water samples

To analyze total phosphorus (TP) and nitrogen (TN), the lake water was first digested with persulfate solution (APHA 1995) and then analyzed using a continuous flow analyzer (QuAAtro, BL-TEC) (Nøjiri 1987; Otsuki et al. 1993) as PO₄-P and NO₃-N, respectively. The water samples for POC/N were filtered onto precombusted (450°C for 4 h) GF/F glass-fiber filters (Whatman) and the filters were dried at 100°C. The samples were stored in a desiccator until isotope measurement.

### Benthic samples

*Chironomus plumosus* larvae and oligochaetes were sorted using a stereomicroscope. For stable isotope analysis, the larvae and oligochaetes were placed in filtered lake water overnight to clear guts, and then freeze-dried. The samples of relatively large individuals were homogenized using scissors in a microtube, whereas the samples of small individuals were not homogenized. Samples were stored in a desiccator until isotope measurement. For biomass analysis, after sorting of benthic macroinvertebrates, *C. plumosus* larvae were pooled and weighed wet after ethanol fixation, and oligochaetes were pooled and weighed wet without any fixation. Replicate samples in each station were averaged, and the biomass of *C. plumosus* and oligochaetes were expressed as g-wet m⁻². Surface sedimentary organic matter samples were freeze-dried, treated with HCl fumes for 24 h to remove inorganic carbon, dried again, and stored in a desiccator until stable isotope analysis.
For grain size analysis, sand-size > 63 \mu m and fine-grained fractions < 63 \mu m were measured. Sediment samples were sieved with a 63 \mu m mesh to separate the sand and fine-grained fractions. Dry weights of the two fractions were measured, and the composition of < 63 \mu m grain size was defined as silt composition. The CH4 concentration in the surface sediments was determined using a GC-FID (GC-8A, Shimadzu). To calculate the Bunsen solubility coefficients, temperatures of bottom waters at sampling and room temperature at measurement of CH4 were used.

Carbon and nitrogen isotope composition analysis

The C and N isotope composition of C. plumosus larvae, oligochaetes, POM, and sedimentary organic matter were measured with a continuous-flow isotope ratio mass spectrometer coupled with an elemental analyzer (CF/IRMS system: Flash EA 1112/ConFlo III/Delta plus Advantage, Thermo Finnigan). Stable isotope ratios of C and N were expressed in δ notation (i.e., Eqs. 1, 2) as the difference in parts per thousand (‰) from the Vienna Pee Dee Belemnite (VPDB) standard and atmospheric N2 (i.e., δ15N = 0‰), respectively:

\[
\delta^{13}C = \left[\frac{\text{[13C/12C]_{sample}}}{\text{[13C/12C]_{standard}}} - 1\right] \times 1000
\]  

\[
\delta^{15}N = \left[\frac{\text{[15N/14N]_{sample}}}{\text{[15N/14N]_{standard}}} - 1\right] \times 1000.
\]

We used three kinds of amino acids: DL-(α)-alanine (δ13C = -23.47‰, δ15N = -1.66‰), L-alanine (δ13C = -19.24‰, δ15N = -2.30‰), and glycine (δ13C = -28.80‰, δ15N = +2.55‰) as the internal laboratory standards. Repeated analysis of the laboratory standard indicated an analytical precision of 0.15‰ for δ13C and 0.32‰ for δ15N.

Estimation of methane-derived carbon contribution and methanogen-derived biomass

To estimate the contribution of methane-derived carbon to C. plumosus larvae and oligochaetes, a simple two-source isotope mixing model was used (Phillips and Gregg 2001), assuming that POM and methane-oxidizing bacteria were end-members:

\[
\delta X = f_{\text{POM}} (\delta X_{\text{POM}} + \Delta X) + f_{\text{MOB}} (\delta X_{\text{MOB}} + \Delta X) \quad (3)
\]

\[
f_{\text{POM}} + f_{\text{MOB}} = 1 \quad (4)
\]

where δX was δ13C of macroinvertebrates (‰), fPOM and fMOB were contributions of POM and methane-oxidizing bacteria, \(\delta X_{\text{POM}}\) and \(\delta X_{\text{MOB}}\) were δ13C of POM and methane-oxidizing bacteria (‰), and ΔX was fractionation of macroinvertebrates (‰). The fractionation of macroinvertebrates (ΔX) was set as +0.8‰ (DeNiro and Epstein 1978; Vander Zanden and Rasmussen 2001). The δ13C of methanogen-derived bacteria (δXMOB) was calculated by δ13C of biogenic CH4 in the sediment of Lake Kasumigaura (-81.6 to -58.7‰, n = 50; Kohzu and Imai 2015), and a fractionation factor between CH4 and methane oxidizing bacteria (-39 to -3‰) (Summons et al. 1994; Templeton et al. 2006), resulting in \(\delta X_{\text{MOB}} = -120.6 \text{ to } -61.7‰\). We show methane-derived carbon contributions estimated using \(\delta X_{\text{MOB}} = -85.7‰\) calculated by average δ13C of CH4 (-69.7‰), and the fractionation value of -16‰ which has been widely used in previous studies (e.g., Jones et al. 2008; Agasild et al. 2018). We also discuss a range of probable methane-derived carbon contributions using the minimum (-81.6 + -39 = -120.6‰) and maximum (-58.7 + -3 = -61.7‰) values. The fMOB was defined as methane-derived carbon contribution to benthic macroinvertebrates in the present study.

Previous studies have used sedimentary organic matter and methanogen-oxidizing bacteria as food sources for the calculation of methane-derived carbon contributions (e.g., Jones et al. 2008; Agasild et al. 2018). In the present study, we examined whether POM or sedimentary organic matter was the main phytoplankton-derived food sources for C. plumosus and oligochaetes based on the δ13C and δ15N values (Fig. 3). The δ13C values of POM (10.5 ± 1.2‰, 10.5 ± 1.3‰, 11.8 ± 2.6‰, and 11.2 ± 1.2‰ at Sta. 3, 7, 9, and 12, respectively) were higher than those of sedimentary organic matter (7.3 ± 0.4‰, 7.1 ± 0.6‰, 7.5 ± 0.4‰, and 7.6 ± 1.3‰ at Sta. 3, 7, 9, and 12, respectively). Assuming isotope fractionation of 13C and 15N from food sources to C. plumosus and oligochaetes was 0.8‰ (DeNiro and Epstein 1978; Vander Zanden and Rasmussen 2001) and 3.3‰ (Minagawa and Wada 1984; Wada et al. 1987), respectively, δ15N values of POM could explain the isotopic signature of the benthic macroinvertebrates, whereas sedimentary organic matter alone could not explain the δ15N isotopic signatures (Fig. 3). Therefore, we assumed that POM was the main phytoplankton-derived carbon food source for these benthic macroinvertebrates, and calculated methane-derived carbon contributions to benthic macroinvertebrates using POM values in the present study. Methane-derived biomass of C. plumosus and oligochaetes in each month were estimated by multiplying their biomass (g-wet m⁻²) and mean of methane-derived carbon contribution (%).

Relationship between methane-derived carbon variation and environmental variables

Multiple linear regressions were employed to assess which environmental variables were associated to methane-derived carbon contributions to C. plumosus and oligochaetes. The analysis used water temperature in the bottom layer, DO saturation and stations as independent variables, and methane-derived carbon contributions as a dependent variable, monthly means of each variable were used. The methane-derived carbon contributions were arcsine square root-transformed to improve normality (Shapiro–Wilk test). Stations were transformed to dummy variables as they were categorical data. Since DO (mg L⁻¹) was significantly
negatively correlated to water temperature \((n = 52, R^2 = 0.77, p < 0.001)\), we used DO saturation (%) to avoid multicollinearity in multiple linear regressions. The relationship between methane-derived carbon contributions and CH\(_4\) concentration were not examined directly since CH\(_4\) concentrations in pore water of sediments were not collected simultaneously with benthic macroinvertebrate samples. Thus, if differences in methane-derived carbon contributions were observed among stations, it was assumed that differences in sediment characteristics, including CH\(_4\) concentrations, could be significant explanation factors. Since C. plumosus larvae are known to be inactive and rarely feed at temperatures < 5°C (Hilsenhoff 1966), we conducted two patterns of multiple linear regression analyses: (1) all data were used and (2) data excluding samples of January and February when water temperature was < 5°C were used. Analysis was conducted using R software (version 3.6.3) and “car” package for ANOVA (type II) (Fox and Weisberg 2019).

**Results**

**Environmental variables**

Although TN concentrations in upper stream stations (Sta. 3 and 7) were higher than those in lower stream stations (Sta. 9 and 12) (Table 1; \(p < 0.05\), Steel-Dwass’s test), there were no significant difference in transparency and TP concentration among stations (Table 1; \(p > 0.05\), Steel-Dwass’s test). In contrast to surface-water quality, sediment characteristics were significantly different among stations. CH\(_4\) concentrations at Sta. 3 and 9 were significantly higher than those at Sta. 7 and 12 (Table 1, see Supporting Information Fig. S1). CH\(_4\) concentrations at Sta. 3, 7, and 9 increased in summer and autumn. Sediment grains were mostly < 63 μm at Sta. 3 (98.5%) and Sta. 9 (99.2%), and low at Sta. 12 (6.87%). The composition at Sta. 7 showed an intermediate value (78.2%). Sediment compositions were considered as silt at Sta. 3 and 9, silt to sand at Sta. 7, and sand at Sta. 12.

Water temperature varied from 3.6°C to 30.3°C throughout the year (Fig. 4). Maximum temperature was observed in July or August, and the minimum was observed in February at each station. In summer, weak stratification (\(\Delta\)water temperature \(\approx 0.5–2.8°C\)) occurred. Stratification led to lower DO concentration in the bottom layer (Fig. 4). The minimum DO concentration was not lower than 2.0 mg L\(^{-1}\) during the study period, and at the bottom layer, were not significantly different among stations throughout the year (Table 1).

**Seasonal variations in \(\delta^{13}C\) values of POM, sedimentary organic matter, C. plumosus larvae, and oligochaetes**

\(\delta^{13}C\) values of POM ranged from −28.7 to −20.8‰ at Sta. 3, −29.3 to −23.7‰ at Sta. 9, −29.5 to −23.4‰ at Sta. 7, and
−28.7 to −25.4‰ at Sta. 12 (Fig. 5). The δ¹³C values of POM were relatively high in spring and summer, and low in winter at Sta. 3, 7, and 9, whereas there was no seasonal trend of δ¹³C values of POM at Sta. 12. The δ¹³C values of sedimentary organic matter were different among stations (Table 1; p < 0.05, Steel-Dwass’s test); highest in Sta. 7 (−23.6 ± 0.3‰) and lowest in Sta. 12 (−26.4 ± 0.3‰). Variations of δ¹³C of sedimentary organic matter within each station were relatively small, and standard deviations were 0.3–0.4‰ at all stations.

At Sta. 3, δ¹³C values of C. plumosus increased from May to July (Fig. 5). From August, the δ¹³C values were lower than those of POM, and the lowest value was −47.6‰ in September. Relatively lower values were also observed in winter. At Sta. 9, clear seasonal variation of δ¹³C of C. plumosus was observed, and the δ¹³C values were depleted from September to November, showing the minimum value of −48.5‰. At Sta. 7 and 12, there were no ¹³C-depleted C. plumosus, and the δ¹³C values varied, synchronizing with those of POM.

At Sta. 3, δ¹³C values of oligochaetes were lower than those of POM throughout the year (Fig. 5). In addition, ¹³C-depleted individuals were occasionally observed, especially in winter, showing a minimum value of −45.4‰ in March. At Sta. 9, δ¹³C values of oligochaetes were always lower than those of POM except for winter, and the minimum δ¹³C value was −34.6‰ in April 2017. At Sta. 7 and 12, there were no seasonal variations of δ¹³C of oligochaetes, and the average values of δ¹³C were −26.0 ± 0.9‰ and −26.4 ± 0.7‰, respectively.

Relationship between δ¹³C and δ¹⁵N values of C. plumosus larvae and oligochaetes

At both Sta. 3 and 9, significantly positive correlations between δ¹³C and δ¹⁵N were found in C. plumosus and oligochaetes (p < 0.001, Fig. 3). At Sta. 7, a significantly positive correlation between δ¹³C and δ¹⁵N was observed in oligochaetes (p < 0.01), whereas no correlations were observed in the others (C. plumosus at Sta. 7 and 12, and oligochaetes at Sta. 12).
Fig. 5. Temporal variations of δ¹³C values of *Chironomus plumosus* larvae (box plot; left), oligochaetes (box plot; right), and particulate organic matter (asterisk; red) at Sta. 3 (a, e), 9 (b, f), 7 (c, g), and 12 (d, h) in Lake Kasumigaura from April 2017 to April 2018.
Fig. 6. Seasonal variations in density of (a) Chironomus plumosus larvae and (b) oligochaetes, biomass of (c) C. plumosus and (d) oligochaetes, contributions of methane-derived carbon to (e) C. plumosus and (f) oligochaetes, and methane-derived biomass of (g) C. plumosus and (h) oligochaetes at Sta. 3, 9, 7, and 12 in Lake Kasumigaura from April 2017 to April 2018.
Density and biomass of *C. plumosus* larvae and oligochaetes

The density and biomass of *C. plumosus* at Sta. 3 reached a maximum of 326 inds m⁻² and 6.3 g-wet m⁻², respectively, in June and decreased in autumn and winter (Fig. 6a,c). *C. plumosus* at Sta. 7 increased from May to August, showing 256 ± 182 inds m⁻² and 2.9 ± 1.0 g-wet m⁻², respectively, and low density and biomass were observed during other months. At Sta. 9, the density and biomass of *C. plumosus* showed a maximum of 548 inds m⁻² and 7.1 g-wet m⁻², respectively, in July. Although the density of *C. plumosus* at Sta. 12 showed relatively high values in June, December, and April, there were no clear seasonal variations in the biomass.

Oligochaetes at Sta. 3 showed a maximum of density (7.9 x 10³ inds m⁻²) and biomass (4.6 g-wet m⁻²) in June as well as *C. plumosus*; however, the density and biomass were relatively stable during the other months, showing 1.3 ± 0.7 x 10³ inds m⁻² and 1.1 ± 0.6 g-wet m⁻², respectively (Fig. 6b,d). At Sta. 7, oligochaetes were relatively stable in density and biomass and showed 1.4 ± 0.8 x 10³ inds m⁻² and 0.81 ± 0.52 g-wet m⁻², respectively, throughout the year. Density and biomass of oligochaetes at Sta. 9 decreased in summer but showed relatively higher values in autumn and winter. At Sta. 12, there were no clear seasonal variations in density and biomass of oligochaetes as well as those of *C. plumosus*. The biomass of oligochaetes (0.66 ± 0.33 g-wet m⁻²) was higher than that of *C. plumosus* (0.20 ± 0.22 g-wet m⁻²) at Sta. 12.

**Methane-derived carbon contribution and methane-derived biomass**

At Sta. 3, methane-derived carbon contributions of *C. plumosus* were quite low from May to July (Fig. 6e). The contribution increased from August and showed the highest value of 38% in September. Relatively high values were observed even in winter. At Sta. 9, the methane-derived carbon contribution of *C. plumosus* showed clear seasonal variations, where higher values observed in autumn, and reaching the highest value of 38% in November. At Sta. 7 and 12, the methane-derived carbon contributions of *C. plumosus* were relatively low (0.3% ± 0.9% and 0.6% ± 1.2%, respectively) throughout the year. The methane-derived carbon contributions at Sta. 3 and 9 were significantly higher than those at Sta. 7 and 12 (Steel-Dwass’s test, *p* < 0.01; Fig. 7), and there were no significant differences in methane-derived carbon contributions between Sta. 3 and 9, and Sta. 7 and 12 (Steel-Dwass’s test, *p* > 0.05; Fig. 7).

The methane-derived carbon contribution to oligochaetes at Sta. 3 remained around 10% throughout the year, and relatively high contributions were observed in winter (~32%; Fig. 6f). At Sta. 9, a relatively high methane-derived carbon contribution to oligochaetes (~16%) was observed in April. The contribution decreased in winter, and then increased again in spring. At Sta. 7, the methane-derived carbon contribution to oligochaetes was low throughout the year, but occasionally the contribution reached up to 9.1% in spring. At Sta. 12, the contributions to oligochaetes were quite low, and relatively negligible throughout the year. The methane-derived carbon contributions were significantly different among stations (Steel-Dwass’s test, *p* < 0.01 for all combinations except for between Sta. 7 and 12 [*p* < 0.05]; Fig. 7).

The multiple linear regression analysis demonstrated that methane-derived carbon contributions to *C. plumosus* were significantly explained by station only, and water temperature and DO saturation were not significant variables (Table 2). There was no difference in significance between the different data set of *C. plumosus* (all data vs. excluding data of < 5°C). Whereas, methane-derived carbon contributions to oligochaetes were

**Table 2.** Summary of multiple linear regressions and ANOVA examining the influence of environmental variables on methane-derived carbon contributions to *Chironomus plumosus* larvae and oligochaetes. *C. plumosus* (1) and (2) indicate all data and the data excluding January and February, when water temperature was lower than 5°C, respectively. Significant outcomes are highlighted in bold.

| Source of variation | df | *F* | *p* | *F* | *p* | *F* | *p* |
|---------------------|----|-----|-----|-----|-----|-----|-----|
| Water temperature   | 1  | 0.136 | 0.715 | 0.41 | 0.524 | 9.74 | <0.01 |
| DO saturation       | 1  | 0.716 | 0.403 | 1.39 | 0.246 | 0.005 | 0.946 |
| Station             | 3  | 16.8 | <0.001 | 20.0 | <0.001 | 30.0 | <0.001 |
significantly explained by water temperature and stations (Table 2).

Methane-derived biomass of C. plumosus peaked in August (0.30 g-wet m$^{-2}$) at Sta. 3 and in September (0.86 g-wet m$^{-2}$) at Sta. 9 (Fig. 6g), whereas biomass of C. plumosus was relatively small, although showing peaks in summer (0.0018 g-wet m$^{-2}$ at Sta. 7 and 0.0021 mg m$^{-2}$ at Sta. 12). Clear seasonal variations in methane-derived biomass of oligochaetes at Sta. 3 and 9 were not observed (Fig. 6h). The average values of the biomass at Sta. 3 and 9 were 0.12 ± 0.09 g-wet m$^{-2}$ and 0.13 ± 0.10 g-wet m$^{-2}$, respectively. The methane-derived biomass of oligochaetes was equivalent to or higher than those of C. plumosus throughout the year except for summer at Sta. 3 and 9. The methane-derived biomass of oligochaetes at Sta. 12 was negligibly small as well as that of C. plumosus. Whereas, at Sta. 7, the biomass of oligochaetes showed slightly high values, 0.021 ± 0.027 g-wet m$^{-2}$, from April to October.

**Discussion**

The δ$^{13}$C of C. plumosus showed a large fluctuation range (~48.5 to ~20.1‰), resulting in large spatial variations of methane-derived carbon contributions to C. plumosus in Lake Kasumigaura (0–38%; Figs. 5, 6). As to δ$^{13}$C spatial variabilities of benthic macroinvertebrates within a lake in previous studies, the δ$^{13}$C values of C. plumosus in macrophyte-dominated habitats were lower than those in open-water, plankton-dominated habitats in a polymictic and eutrophic lake (Võrtsjärv; Agasild et al. 2018). The δ$^{13}$C values of C. anthracinus and C. plumosus were lighter in deeper stations than those in shallower stations in Esthwaite Water and Wyresdale Park Lake (Grey et al. 2004). These two previous studies suggested that lower DO concentrations above the sediment surface accelerated the methane oxidation and incorporation of methane-derived carbon by chironomid larvae. In the present study, methane-derived carbon contributions to C. plumosus were significantly explained by stations, and not by either water temperature or DO saturation (Table 2; Fig. 7). Among stations, there were large and significant differences in sediment characteristics such as ignition loss, TOC, silt composition, and CH$_4$ concentration (Table 1). Although direct analysis between CH$_4$ and benthos samples was not possible, the CH$_4$ concentrations in 2019 were clearly different among sites, and higher at Sta. 3 and 9, where methane-derived carbon contributions were highest. Higher CH$_4$ concentration in sediments correlates with higher silt composition and organic carbon concentrations (e.g., Fedorov et al. 2019). Therefore, the results suggest that spatial variations of methane-derived carbon contributions to C. plumosus were regulated by CH$_4$ concentration or case 1 control.

The methane-derived carbon contributions to C. plumosus at Sta. 3 and 9 showed clear seasonal variation and relatively high values in summer and autumn, similar to previous studies conducted in polymictic lakes (Yasuno et al. 2012; Agasild et al. 2018). High methane-derived carbon contributions are explained by Agasild et al. (2018) discussing lower DO induced by dense macrophyte vegetation, and Yasuno et al. (2012) discussing higher CH$_4$ concentrations. In the present study, since no relationship between DO concentration in bottom waters and methane-derived carbon contributions were observed, seasonal variations of methane-derived carbon contribution to C. plumosus should also be regulated by CH$_4$ concentrations, supporting a case 1 scenario (Fig. 1). CH$_4$ concentration in pore water in the upper 10 cm of bottom sediment showed the highest values from summer to autumn in Lake Kasumigaura (Sta. 3 and 9; Nakamura et al. 1999), which was consistent with the seasonal variations of methane-derived carbon contributions to C. plumosus in the present study.

Seasonal variations in stable isotopic ratios can be induced when larger, more δ$^{13}$C-depleted larvae pupate and emigrate from the population (Grey et al. 2004). A previous study in Lake Kasumigaura observed a significant emergence from April to June (Iwakuma 1992). In the present study, increase in δ$^{13}$C values of C. plumosus occurred from May to July at Sta. 3 and 9 (Figs. 5, 6), suggesting synchronized pupation (emigration of δ$^{13}$C-depleted individuals from the population) also affected the seasonal δ$^{13}$C dynamics, especially in terms of an increase in mean δ$^{13}$C values within a population.

The δ$^{13}$C of oligochaetes showed large fluctuation range (~45.4 to ~23.6‰), resulting in large spatial variations of methane-derived carbon contributions to oligochaetes in Lake Kasumigaura as well as C. plumosus (0–32%; Figs. 5, 6). Hershey et al. (2006) reported that δ$^{13}$C of oligochaetes in offshore sites was lighter about 5‰ than that of surficial sediments and seston, whereas there was no difference in δ$^{13}$C values of oligochaetes, surficial sediments, and seston in nearshore sites. In the present study, the difference of δ$^{13}$C between POM and oligochaetes was up to ~17.7‰ at Sta. 3 (Fig. 5). The methane-derived carbon contributions to oligochaetes at Sta. 3 and 9 (median = 8.9% and 5.7%, respectively) were almost equivalent to those of C. plumosus (median = 4.7% and 7.6%), confirming that oligochaetes were also fueled by methane-derived carbon (Fig. 7).

The multiple linear regression analysis revealed the methane-derived carbon contributions to oligochaetes were significantly explained by stations and water temperature in the bottom waters (Table 2). Since station was a significant explanatory variable, spatial variations of the methane-derived carbon contributions to oligochaetes could be regulated by CH$_4$ concentration (case 1) as well as C. plumosus. Although water temperature was statistically significant (p < 0.01), relatively lower methane-derived carbon contribution during winter may have affected the analysis (Fig. 6f). However, in contrast to the case of C. plumosus, the methane-derived carbon contributions to oligochaetes did not clearly increase in summer and autumn although CH$_4$ concentrations were relatively high during the seasons (Supporting Information Fig. S1 and Nakamura et al. 1999), and the seasonal variations of
methylene-derived carbon contributions to oligochaetes were obviously less than that of *C. plumosus* (Figs. 5, 6). The results suggest that the methanee-derived carbon contributions to oligochaetes were seasonally controlled by case 4 (ecological characteristics). *C. plumosus* filter feeds by drawing overlying oxygenated water through its burrow (Matisoff et al. 1985), leading to higher methane oxidizing bacteria production in their burrows. Whereas, oligochaetes have a lower ability to pump oxygenated water into their tubes than Chironomidae larvae (Zhang et al. 2010). However, oligochaetes ingest sediment particles from the reducing zone of the sediment (Matisoff et al. 1985), which suggests that consumption of methane oxidizing bacteria was comparatively consistent although the methane oxidizing bacteria production in their tubes was not as large as that of *C. plumosus*.

Although the sediment characteristics were similar at Sta. 3 and 9, the methanee-derived carbon contributions to oligochaetes were different between the two stations, especially in winter; there were no $^{13}$C-depleted oligochaetes at Sta. 9, but $^{13}$C-depleted oligochaetes were found at Sta. 3. The surface sediment CH$_4$ concentration in winter at Sta. 3 was significantly higher than that of Sta. 9 (Supporting Information Fig. S1). This suggests the highest methanee-derived carbon contribution in winter with higher CH$_4$ concentration at Sta. 3, supporting case 1 predominated in winter (Fig. 1). Stable isotopic compositions of oligochaetes were measured without species identification in the present study. Previous studies showed a total of 13 species of oligochaetes were observed in Lake Kasumigaura (Ohtaka 2014), and their life cycles and ecological behaviors were different among species (e.g., Carroll and Dorris 1972; Yasuda and Okino 1987; Ohtaka 1992; Ohtaka and Kikuchi 1997), suggesting interspecific differences may affect variability of $\delta^{13}$C and $\delta^{15}$N. In future studies, interspecific differences of methanee-derived carbon contributions should be examined to understand ecological roles of oligochaetes in methane dynamics.

In the present study, methanee-derived carbon contributions were estimated by a two-source mixing model using the average $\delta^{13}$C values of CH$_4$ observed in Lake Kasumigaura and a fractionation value between CH$_4$ and methane-oxidizing bacteria of $-16$‰, which has been widely used to estimate methanee-derived carbon contributions to benthic macroinvertebrates in previous studies (e.g., Jones and Grey 2004; Deines and Grey 2006; Jones et al. 2008; Agasild et al. 2018). However, there were highly variable $\delta^{13}$C values of biogenic CH$_4$ ($\delta^{13}$C$_{\text{CH}_4} = -81.6$ to $-58.7$‰; Kohzu and Imai 2015) and fractionation factors for the methanotrophic fractionation ($\Delta\delta^{13}$C$_{\text{CH}_4-MOB} = -39$ to $-3$‰; Summons et al. 1994; Templeton et al. 2006). Therefore, using these maximum and minimum values, ranges of probable methanee-derived carbon contributions were estimated (Table 3). The results showed that methanee-derived carbon contributions to *C. plumosus* and oligochaetes were estimated up to 64.2% and 54.4% in the upper estimation ($\delta^{13}$C$_{\text{CH}_4} = -58.7$‰ and $\Delta\delta^{13}$C$_{\text{CH}_4-MOB} = -3$‰), respectively, and up to 24.1% and 18.8% in the lower estimation ($\delta^{13}$C$_{\text{CH}_4} = -81.6$‰ and $\Delta\delta^{13}$C$_{\text{CH}_4-MOB} = -39$‰), respectively (Table 3). The analysis shows that methanee-derived carbon accounted for < 15% of carbon in *C. plumosus* and oligochaetes biomass at Sta. 7 and 12, where CH$_4$ concentration is low. On the other hand, methanee-derived carbon can potentially contribute up to 64% of the benthos carbon biomass at Sta. 3 and 9, where CH$_4$ concentration is high.

Contrary to methanee-derived carbon contribution to oligochaetes (%), methanee-derived biomass (i.e., methanee-derived carbon contributions × biomass) from December to April were 0.10 ± 0.10 g-wet m$^{-2}$ at Sta. 3 and 0.11 ± 0.10 g-wet m$^{-2}$ at Sta. 9 with no significant difference (Fig. 6f,h). The result suggests that although there were some individuals which showed high methanee-derived carbon contributions, the ecological impact of oligochaetes on methane-fueled lake foodwebs was similar at both stations.

Methanee-derived biomass of *C. plumosus* at Sta. 3 and 9 showed relatively high values during summer and autumn (Fig. 6g). The data suggest that transfer of methanee-derived carbon to higher trophic organisms such as fishes via

| Benthos       | $\delta^{13}$C$_{\text{CH}_4}$ (%) | $\Delta\delta^{13}$C$_{\text{CH}_4-MOB}$ (%) | Sta. 3       | Sta. 9       | Sta. 7       | Sta. 12      |
|--------------|-------------------------------|------------------------------------------|--------------|--------------|--------------|--------------|
| *C. plumosus*| $-58.7$                        | $-3$                                     | 0–63.3 (15.6)| 0–64.2 (15.8)| 0–8.1 (0.4)  | 0–10.6 (1.0) |
|              | $-69.7$                        | $-16$                                    | 0–38.0 (9.4) | 0–37.9 (9.5) | 0–4.9 (0.3)  | 0–6.4 (0.6)  |
|              | $-81.6$                        | $-39$                                    | 0–24.1 (6.0) | 0–23.8 (6.0) | 0–3.2 (0.2)  | 0–4.1 (0.4)  |
| Oligochaetes | $-58.7$                        | $-3$                                     | 0–54.4 (14.9)| 0–26.4 (9.7) | 0–14.9 (3.5) | 0–7.8 (1.3)  |
|              | $-69.7$                        | $-16$                                    | 0–31.8 (9.0) | 0–15.8 (5.8) | 0–9.1 (2.1)  | 0–4.7 (0.8)  |
|              | $-81.6$                        | $-39$                                    | 0–18.8 (5.7) | 0–10.0 (3.7) | 0–5.8 (1.3)  | 0–3.0 (0.5)  |

*Table 3.* Variations of methanee-derived carbon contributions to *Chironomus plumosus* and oligochaetes depending on $\delta^{13}$C values of CH$_4$ ($\delta^{13}$C$_{\text{CH}_4}$) and fractionation factors between CH$_4$ and methane-oxidizing bacteria ($\Delta\delta^{13}$C$_{\text{CH}_4-MOB}$) at Sta. 3, 9, 7, and 12 in Lake Kasumigaura during the study period.
C. plumosus may be larger in summer and autumn, whereas the methane-derived carbon transfer might be limited in winter and spring. On the other hand, the methane-derived biomass of oligochaetes at Sta. 3 and 9 showed no clear seasonal variations (Fig. 6h). In other words, the methane-derived biomass of oligochaetes showed relatively high values even when that of C. plumosus was relatively low. For example, the methane-derived biomass of C. plumosus during the winter at Sta. 3 was quite low (≈ 0.019 g-wet m⁻²), whereas the combined methane-derived biomass of two macroinvertebrates during the same period showed 0.041–0.093 g-wet m⁻². The complementary variations of two major benthic macroinvertebrates might enhance opportunities for higher trophic levels to assimilate the methane-derived carbon in this lake throughout the year.

In conclusion, we examined spatial and seasonal variations of methane-derived carbon contributions to C. plumosus and oligochaetes, and factors affecting the methane-derived carbon contributions in shallow, eutrophic Lake Kasumigaura. Large spatial and seasonal variability of methane-derived carbon contributions within the lake were clarified, where methane-derived carbon contributions varied spatially along gradients of CH₄ concentration, TOC concentration, and silt composition in the sediment. For both C. plumosus and oligochaetes, the spatial variability of the methane-derived carbon contributions were similar to case 1 (Figs. 1, 7), mainly controlled by CH₄. Seasonal variations of methane-derived carbon contributions to C. plumosus showed that CH₄ should also be an important controlling factor, and agree with a study in Lake Izu-numa (Yasuno et al. 2012). With regards to oligochaetes, we observed case 1 and 4 controls. The difference in methane-derived carbon contributions between Sta. 3 and 9 in winter could be regulated by case 1 control, whereas seasonal variations of methane-derived carbon contributions to oligochaetes compared to C. plumosus suggests a case 4 control (ecological characteristics). The present study revealed that seasonal variations of methane-derived carbon contributions were taxonomically different. The divergence suggests complementary seasonal variations of methane-derived biomass of C. plumosus and oligochaetes, especially at Sta. 3, suggesting relatively high methane-derived carbon could be transferred to higher trophic levels during all seasons. In future studies, transfer of methane-derived carbon to both pelagic and benthic organisms should be investigated to clarify the importance of methane-derived carbon in lacustrine ecosystems.

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