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Evidence of methanogenic activity associated with past fish farming in Lake Soyang, South Korea

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Abstract

In this study, a 54-cm-long sediment core was collected from an area with a history of fish farming to investigate a potential link between methane production and past aquaculture activities in Lake Soyang, a large artificial lake (ca. 2–110 m water depth, 16.08 km^2 in area). Along with bulk elements (carbon and nitrogen), we analyzed specific lipid biomarkers, including *n*-alkanes, sterols, glycerol dialkyl diethers (DGDs), and glycerol dialkyl glycerol tetraethers (GDGTs). Additionally, we measured the compound-specific carbon isotopic composition (δ^{13}C) of specific lipids, excluding GDGTs. Evidence of prior aquaculture activities was indicated by high total organic carbon content (~ 30 wt%) and elevated concentrations of coprostanol and stigmasterol, reflecting substantial deposition of mixed organic matter (OM) from aquaculture practices, such as fish feed and feces. The δ^{13}C values of sterols (-28.4 ± 1.4‰) were relatively enriched compared to those of isoprenoid DGDs, such as archaeol and OH-archaeol (-40.0 ± 3.2‰). This suggests that increased acetoclastic methanogenic activities may be closely linked to the assimilation of by-products derived from aquaculture-related OM as potential carbon sources. Our findings highlight the potential importance of methane production within aquaculture systems and the environmental factors influencing this process. These results could inform the development of systematic management strategies to mitigate methane emissions from aquaculture systems.

Keywords Aquaculture, Organic matter, Methanogenesis, Lipid biomarker, Compound-specific isotope analysis, Eutrophication

1 Introduction

A highly intensive way of fish farming, the aquaculture fish cage system, has been widely used in lakes, reservoirs, rivers, bays, and coastal areas (Naylor et al. 2000; Varol 2019; FAO (Food and Agriculture Organization of the United Nations) 2020; Kim et al. 2000). During fish farming, substantial quantities of organic matter (OM) are supplied as food sources for fish species within cage systems (McGhie et al. 2000; Urbina 2016). These farming systems release considerable amounts of particulate organic waste, including unconsumed feed, feces, and metabolic excretion products. These can accumulate in the underlying and surrounding sediments (Aguado-Gimenez and Garcia-Garcia 2004; Yokoyama et al. 2006; Mayor et al. 2017). The accumulated wastes rich in organic carbon, phosphorus, and nitrogen may substantially alter the sedimentary environments, such as the sedimentary redox conditions near the fish cages (Wildish et al. 2001; Tomassetti and Perrello 2005; Matijevic

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et al. 2008; Varol 2019). Accumulated organic wastes may continuously induce the deterioration of environmental conditions. This includes increasing turbidity, altering the benthic community structure, and producing methane and hydrogen sulfide (Pitta et al. 2006; Degefu et al. 2011). Therefore, with the increase in fish consumption worldwide (FAO (Food and Agriculture Organization of the United Nations) 2020), there has been increasing concern about the negative impacts on water and sediment quality near aquaculture systems.

Aquaculture-dominated lakes have the potential to contribute to carbon emissions through by-products accumulation from aquaculture activities (Boyd et al. 2007, 2010). With the expansion of lake aquaculture systems, various OMs accumulated in sediments can be remineralized under anaerobic conditions (Green et al. 2012; Naeher et al. 2014; Wang et al. 2017). The microbial activities under anaerobic conditions in the lake sediment can cause the production of greenhouse gases such as methane which are released into the atmosphere (Bastviken et al. 2004; Naeher et al. 2014; Yang et al. 2020). Methane is a greenhouse gas that is > 34 times more potent than carbon dioxide (Wuebbles and Hayhoe 2002; Etminan et al. 2016). Therefore, the intensive aquaculture activities conducted within lakes can be regarded as a potentially important source of methane emissions (Bastviken et al. 2011; Wang et al. 2017; Sabu et al. 2022). Therefore, the methane emissions from aquaculture systems and its severe impact on global climate change are a matter of concern and needs monitoring. However, estimating methane emissions from aquaculture systems is hindered by an incomplete understanding of the microbial mechanisms related to methane cycling, namely production and oxidation. This is because of the complex interactions between OM remineralization and microbial activities.

Lake Soyang is a eutrophic and methane-rich lake in South Korea with a methane production potential of up to 50 μg C m^{-2} d^{-1} (Jin et al. 2016). Since the 1990s, this lake has experienced severe eutrophication because of an excessive accumulation of fish feed and excreta (Kim et al. 1994; Cho et al. 1995; Hwang et al. 1996; Seo 1998; Lee and Lee 2000). In this study, we investigated the vertical variation of specific lipid compounds associated with past fish farming activities in a sediment core. Alongside GDGT abundance, we analyzed lipid biomarkers such as *n*-alkanes, sterols, DGDs, and their carbon isotopic compositions. In this study, we aimed to trace the carbon sources of methane-related microbes and to constrain the linkage between aquaculture-derived OMs and methanogenesis. Based on these events, we assessed important environmental factors affecting the methane cycling in Lake Soyang.

2 Materials and methods

2.1 Core sampling

Aquaculture activities in large reservoirs in South Korea, such as lakes, grew due to industrialization in the early 1980s. However, they have gradually decreased since the 1990s with the discovery that fish feed and excreta cause eutrophication (Heo et al. 1992). Most aquaculture activities in Korean lakes no longer occur, but further adverse effects have been reported from Lake Soyang (Heo et al. 1992; Kim et al. 1994; Cho et al. 1995; Seo 1998; Lee and Lee 2000; Jin et al. 2016). In May 2014, we collected a 54-cm-long sediment core using a gravity corer (129.9761°E, 37.9780°N; Fig. 1). The sediment core was sliced at 1-cm intervals to analyze the bulk elements and lipid biomarkers. Sediment samples were transferred into glass vials and stored at − 20 °C until analysis.

2.2 Radioisotope analysis

The ^{210}Pb activity was indirectly measured using ^{210}Po through the non-destructive method (Nittrouer et al. 1979). This measures radioactive material decay using an alpha spectrometer equipped with low-background silicon surface barrier detectors (PIPS detector, Canberra, USA). This analysis was performed at the Korea Basic Science Institute (KBSI) (Gal et al. 2019). The analytical error was on average 4.0 ± 1.4 Bq/kg (Fig. 2).

2.3 Bulk element analysis

Sediment samples were freeze-dried and homogenized using an agate mortar before conducting bulk element analyses. For TOC, sediment samples were treated with 8 mL of 1 M HCl to remove inorganic carbons. Total nitrogen (TN) analysis was conducted without additional processing. Bulk element analysis, including contents and their isotopic compositions, was performed at Hanyang University using an elemental analyzer connected to an isotope ratio mass spectrometer (IRMS; Isoprime 100, GV Instruments, Manchester, UK). The stable isotopic values of TOC ($\delta^{13}C_{TOC}$) and TN ($\delta^{15}N_{TN}$) were reported in standard delta (δ) notation relative to the Vienna Pee–Dee Belemnite (VPDB) and N_2 AIR scale per mil, respectively.

2.4 Lipid extraction and purification

Sediment samples were extracted using an accelerated solvent extractor (Dionex ASE 200, Dionex Corporation, Sunnyvale, CA, USA) with a solvent mixture of dichloromethane (DCM) and methanol (MeOH) in a 9:1 (v:v) ratio at 100 °C and a pressure of 7.6×10^6 Pa. The extracts were concentrated with a stream of nitrogen and dried with an anhydrous Na_2SO_4 column. For quantification, 5α-androstane, C_{46} GDGT, and C_{22} 7,16-diol were added as internal standards. The total extracts

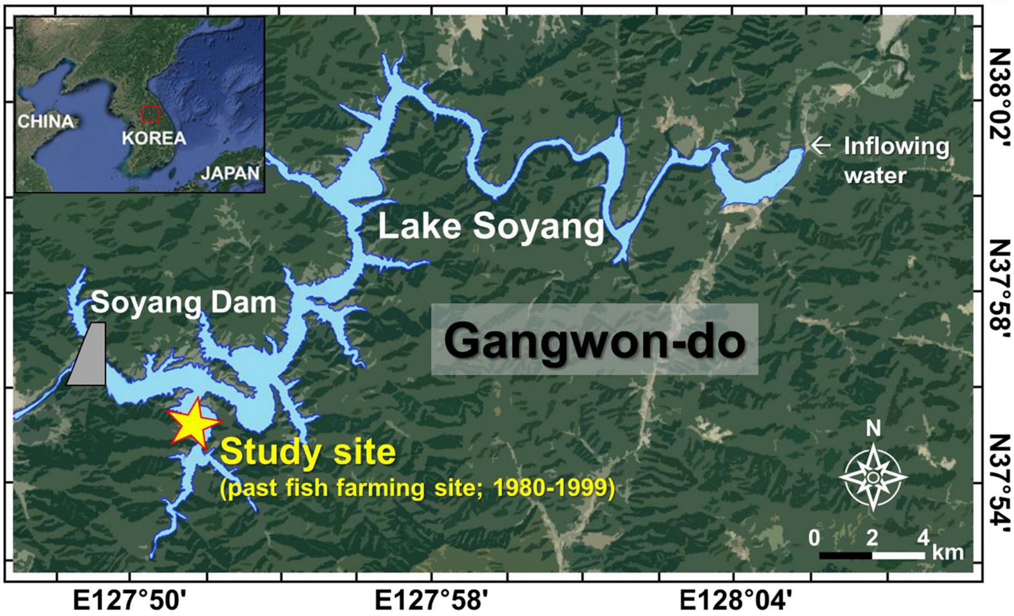

Fig. 1 Map of the study area and location (yellow star) of the sediment core

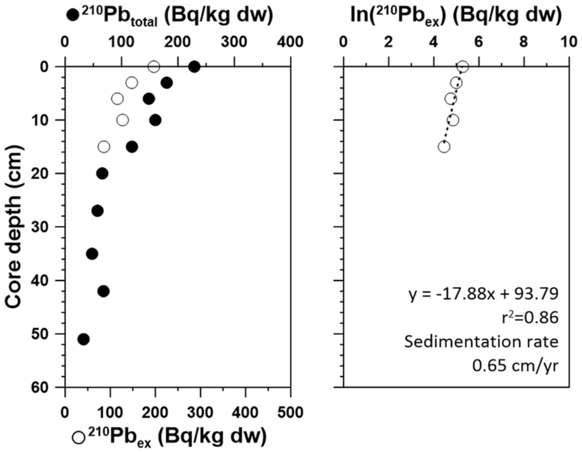

Fig. 2 Downcore profile of $^{210}Pb_{total}$ (filled circles) with the error range and $^{210}Pb_{ex}$ (open circles) in the left panel and ln ($^{210}Pb_{ex}$) (open circles) in the right panel

from all the samples were separated into three fractions over an Al_2O_3 column activated at 150 °C for 2 h. The fractions were eluted sequentially with hexane:DCM (9:1, v:v), hexane:DCM (1:1, v:v), and DCM:MeOH (1:1, v:v), respectively. The apolar fraction (first fraction) was used to detect *n*-alkanes, while the polar fraction (third fraction) was analyzed for several compounds, including sterols, DGDs, and GDGTs. The polar fraction was further divided into two aliquots. One aliquot, containing C_{22} 7,16-diol, was silylated with 25 μL of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and 25 μL of pyridine, and then heated to 60 °C for 20 min to form trimethylsilyl derivatives. The second aliquot, containing C_{46} GDGT, was redissolved in hexane:isopropanol (99:1, v:v) by sonication for 5 min and subsequently filtered through a 0.45 μm polytetrafluoroethylene (PTFE) filter.

2.5 Quantification, identification, and carbon isotope analysis of lipid biomarkers

For quantification, all apolar and silylated polar fractions were analyzed using a gas chromatograph (GC; Shimazu, Kyoto, Japan) equipped with a splitless injector and a flame ionization detector (FID). A fused silica capillary column (CP-sil 5 CB, 25 m length, 0.32 mm internal diameter, 0.12 μm film thickness) was used, with helium (1.3 mL min^{-1}) as a carrier gas. Samples were injected under constant flow at an initial oven temperature of 70 °C. The GC oven temperature was then increased to 130 °C at a rate of 20 °C min^{-1}, followed by a rise to 320 °C at 4 °C min^{-1}, with a final hold time of 15 min. Concentrations were determined by comparing the peak area of each compound to that of 5α-androstane for the apolar fraction and C_{22} 7,16-diol for the polar fraction. Compound identification of the apolar and silylated polar fractions was performed using a GC coupled with a mass spectrometer (MS; QP2010, Shimazu, Kyoto, Japan) operated at 70 eV (cycle time of 0.9 s, resolution of 1000) over a mass range of m/z 50–800. The samples were analyzed under the same temperature program and capillary column setup as described for GC–FID analysis. Molecular structures were identified by comparing their mass spectral fragmentation patterns and retention times with previously published data.

GDGTs were analyzed using an ultra-high-performance liquid chromatography–atmospheric pressure chemical ionization–mass spectrometer (HPLC–APCI–MS) (1290 Infinity II LC, G6135B MSD, Agilent Technologies, Santa Clara, California, USA) at the Korea Polar Research Institute (KOPRI). Separation was performed on two UHPLC silica columns (BEH HILIC, 150 mm length, 2.1 mm internal diameter, 1.7 μm particle size) fitted with pre-columns (5 mm length, 2.1 mm internal diameter) of the same material, maintained at 30 °C. The injection volume was 1 μL. GDGTs were eluted isocratically with a mobile phase of 82% A and 18% B for 25 min, followed by a linear gradient to 35% B over 25 min, then to 100% B over 30 min, with a final hold for 20 min. Solvent A was hexane, while solvent B was a mixture of hexane and 2-propanol (90:10, v:v). The flow rate was maintained at 0.2 mL min^{-1}, resulting in a total run time of 90 min. The APCI–MS conditions were as follows: nebulizer pressure of 60 psi, vaporizer temperature of 400 °C, drying gas (N_2) flow rate of 6 mL min^{-1} at 200 °C, capillary voltage of − 3.5 kV, and a corona current of 5 μA (~ 3.2 kV). Detection and identification were conducted in single ion monitoring mode for $[M+H]^+$ ions (dwell time 35 ms), following the method described by Schouten et al. (2007). GDGTs were quantified by integrating peak areas and using an internal standard according to Huguet et al. (2006).

The $\delta^{13}C$ values of selected compounds were determined at Hanyang University using an isotope ratio mass spectrometer (IRMS) connected to a GC via a combustion interface (VisION, Isoprime, Manchester, UK). This comprised a glass tube packed with copper oxide (CuO), operated at 850 °C. The samples were subjected to the same temperature conditions and capillary column setup used in the GC–FID and GC–MS analyses. Calibration was performed by injecting several pulses of reference gas (CO_2) with a known $\delta^{13}C$ value at the beginning and end of each sample run. The $\delta^{13}C$ values were expressed as per mil (‰) relative to the VPDB. The $\delta^{13}C$ values were corrected to introduce additional carbon atoms during silylation. The analytical errors were less than ± 0.4‰.

2.6 Statistical analysis

To provide a general view of the variability of specific lipid distributions in the sediment samples, gaps in the dataset were first filled as described by Yunker et al. (2005). When some components were not determined, a value of one-half of the minimum value detected for that variable in the entire dataset was set as the detection limit. These values were then replaced by a random number between zero and the detection limit. The fractional abundances of specific lipids were obtained by normalizing each concentration to the summed concentrations of all lipid compounds. Based on the fractional abundances, principal component analysis (PCA) and hierarchical clustering on principal components (HCPC) were performed using R software version 3.4.2 (package information; FactoMineR, Vienna, Austria).

3 Results

3.1 Sedimentation rate and age model

The measured $^{210}Pb_{total}$ activities ranged from 32.6 to 229.1 Bq/kg of dry weight (dw) (Fig. 2). Excess ^{210}Pb activities ($^{210}Pb_{ex}$) were calculated by subtracting the supported level values ($^{210}Pb_{sup}$) from the total activity ($^{210}Pb_{total}$). The $^{210}Pb_{ex}$ varied between 85.8 and 196.5 Bq/kg dw and decreased logarithmically with core depth. The estimated sedimentation rate calculated from $^{210}Pb_{ex}$ using the constant flux and constant sediment accumulation rate model, based on a slope of the logarithmic regression line, was 0.65 cm/yr ($R^2 = 0.86$). Accordingly, the core covers last 83 years.

3.2 Bulk element compositions

The TOC contents and $\delta^{13}C_{TOC}$ values showed substantial changes along the sediment core, ranging from 2.4 to 30.7 wt% and from − 26.2 to − 23.6‰, respectively (Fig. 3). Similarly, the TN contents and $\delta^{15}N_{TN}$ values varied with the values of 0.3–1.9 wt% and 3.0–5.9‰, respectively (Fig. 3). Both TOC and TN contents were higher for the core depths of 15–24 cm than for the other core depths. $\delta^{13}C_{TOC}$ and $\delta^{15}N_{TN}$ values were considerably lower when TOC and TN contents were highest. Based on the vertical profiles of TOC and TN contents, Unit II was defined to span from

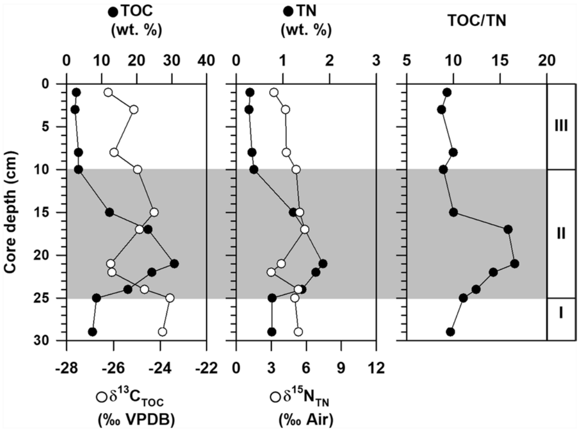

Fig. 3 Downcore profiles of bulk element contents (TOC and TN, filled circles) and their isotopic compositions ($\delta^{13}C_{TOC}$ and $\delta^{15}N_{TN}$, open circles)

25 to 10 cm, encompassing data points where the values reached baseline levels compared to nearby data points. The lower section was designated as Unit I and the upper section as Unit III (Unit I: > 25 cm, Unit II: 25–10 cm, and Unit III: < 10 cm).

3.3 Lipid biomarker characteristics

Representative lipid biomarkers, such as *n*-alkanes, sterols, isoprenoid DGDs, and isoprenoid GDGTs, were detected in core sediments (Fig. 4, Supplementary Information Fig. S1). The high molecular weight (HMW) *n*-alkane concentrations (C_{27} to C_{31}) ranged from 1.2 to 15.6 μg/g dw, in contrast to low concentrations (< 0.7 μg/g dw) of low molecular weight (LMW) *n*-alkanes (C_{17} to C_{21}) (Fig. 4). The concentrations of selected sterols, that is, coprostanol, cholesterol, and stigmasterol, varied between 0.2 and 164.3 μg/g dw. They had overall higher concentrations for Unit II than for other units (Fig. 4). The concentrations of DGDs, namely archaeol and OH-archaeol, also showed increased patterns for Unit II, ranging from 1.0 to 6.3 μg/g dw (Fig. 5). Isoprenoid GDGTs containing zero to three cyclopentane moieties (GDGT-0 to GDGT-3) and crenarchaeol were detected in all the samples (Fig. 5). Isoprenoid GDGTs were dominated by GDGT-0 with the concentrations of 0.1–4.4 μg/g dw, while the other GDGTs (GDGT-1, GDGT-2, and GDGT-3) and crenarchaeol were substantially less abundant (< 0.1 μg/g dw) in all sediments (Fig. 5). In the Unit II section, the $\delta^{13}C$ values for HMW *n*-alkanes and sterols, that is, coprostano and stigmasterol, were − 33.5 ± 1.5‰ and − 28.4 ± 1.4‰, respectively (Fig. 6). The $\delta^{13}C$ values of DGDs ranged from − 44.3 to − 35.5‰, with more depleted values than other lipid compounds (Fig. 6).

3.4 Principal component analysis

PCA was performed on the fractional abundance data of all lipid groups to examine the general variability of total lipid extracts (Supplementary information Fig. S2). For all the core sediments investigated, the first two principal components explained a cumulative 78.6% of the variance. For the first principal component (PC1), explaining 58.6% of the variance, the loading of sterols, isoprenoid DGDs, and isoprenoid GDGTs was the opposite to that of LMW and HMW *n*-alkanes. For the second principal component (PC2), explaining 20.0% of the variance, stigmasterol, archaeol, crenarchaeol, GDGT-1 to 3, and LMW and HMW *n*-alkanes were positively loaded. Meanwhile, OH-archaeol, coprostanol, cholesterol, and GDGT-0 were negatively loaded. The clustering resulted in two groups, showing the Unit III samples as one group separate from the Unit I and II samples.

4 Discussion

4.1 Origins of sedimentary organic matter

The TOC (17.1 ± 9.7 wt%) and TN (1.3 ± 0.5 wt%) contents substantially increased for Unit II (Fig. 3). Based on the age model (sedimentation rate 0.65 cm/yr, see Fig. 2), Unit II corresponds to the period from 1983 to 2002 CE. Considering the uncertainties of the age model,

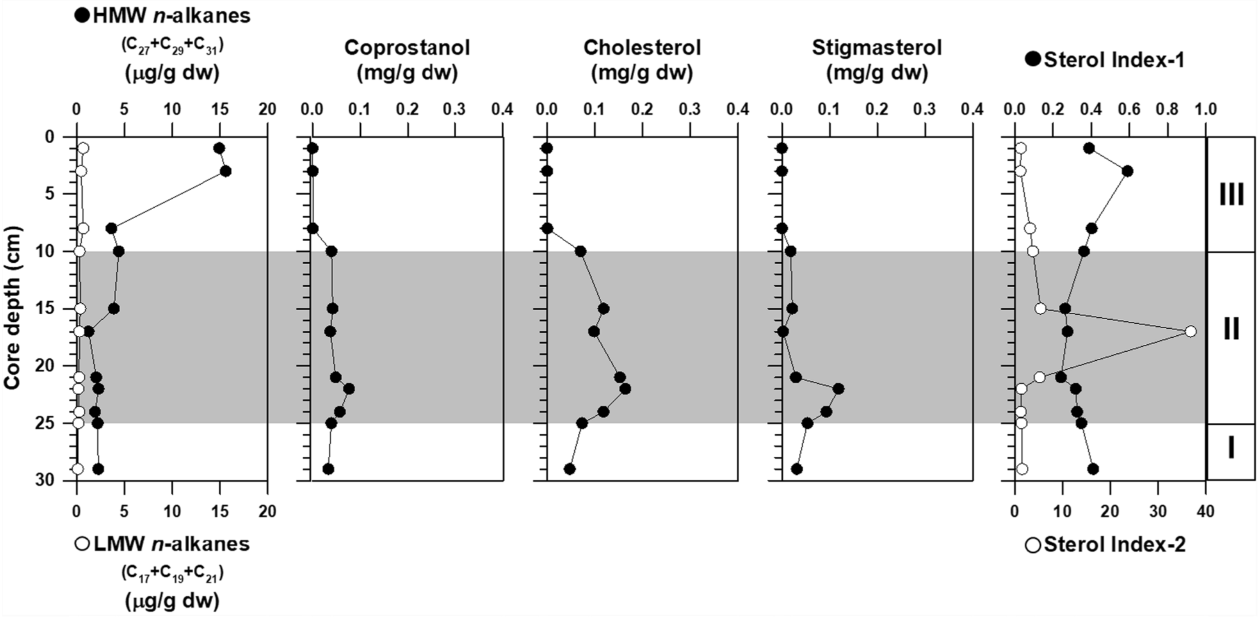

Fig. 4 Downcore profiles of high molecular weight (HMW) *n*-alkanes and sterols, that is, coprostanol, cholesterol, and stigmasterol

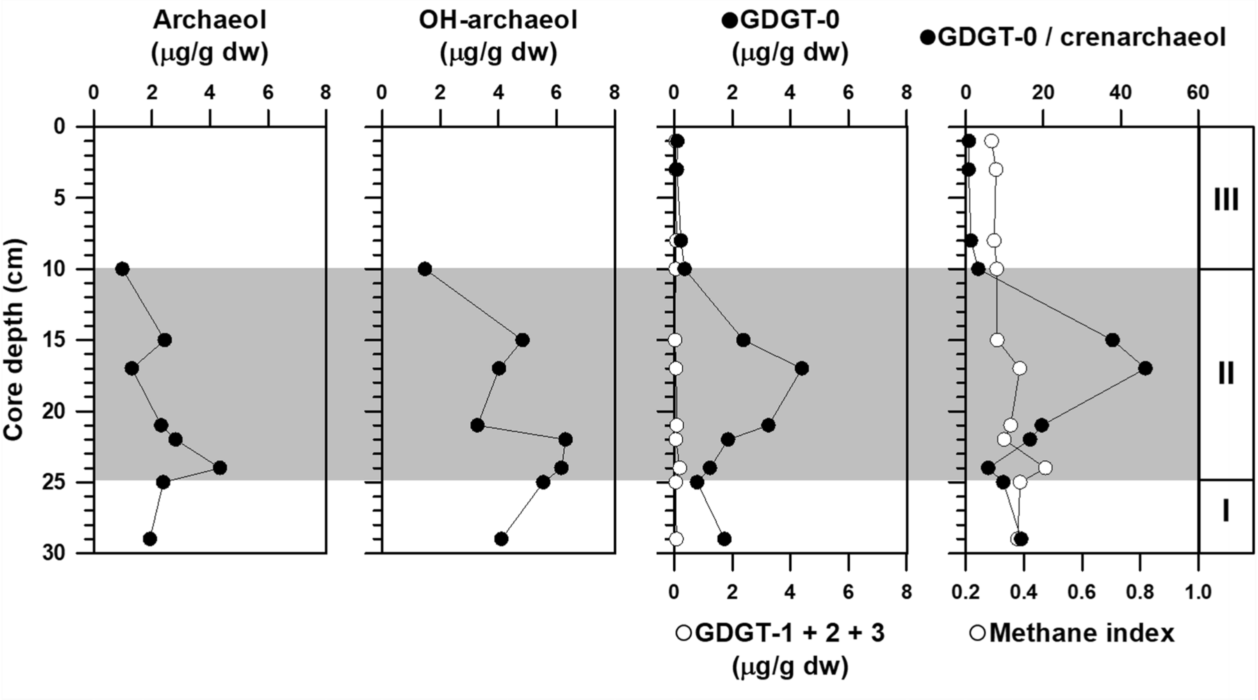

Fig. 5 Downcore profiles of archaeol, OH-archaeol, GDGT-0, and ratios of GDGT-o relative to crenarchaeol (filled circles; Blaga et al. 2009), and the methane index (open circles; Zhang et al. 2011)

this unit corresponds to the fish farming period in Lake Soyang (1980–1999). The increased bulk properties for Unit II were exceptionally high in comparison with other fish farming sites (e.g., Hideaway Bay: 2.7 ± 18.6 wt% for TOC, 0.6 ± 3.4 wt% for TN, Gokasho Bay: 2.1 ± 12.0 wt% for TOC, 0.3 ± 1.8 wt% for TN, and Keban reservoir: 4.9 ± 1.1 wt% for TOC, 0.2 ± 0.1 wt% for TN; McGhie et al. 2000; Yokoyama et al. 2006; Varol 2019). These properties are likely influenced by the massive release of the aquaculture-related OM derived from past fish farming activities in Lake Soyang (Khim et al. 2005). This is in line with intensive fish farming in lake/coastal waters generating large amounts of particulate organic wastes including fish carcass, unconsumed feed, and fecal matter (Yokoyama et al. 2006; Varol 2019). As a possible inference, alongside the predominant carbon content in fish lipids, fish that consume a nitrogen-rich diet may effectively assimilate nitrogen through physiological processes (C/N ratios: 7.3 ± 0.5 for farmed fish bodies, 11.8 ± 0.5 for fish feces; Czamanski et al., 2011). Additionally, considering the different ranges of C/N ratios derived from various OM sources (autochthonous: 4–10 for pelagic organisms, allochthonous: > 12 for terrestrial C_3 plants) (Kendall et al. 2001; Lamb et al. 2006; Wang et al. 2022), another potential explanation for higher TOC contents could be the mixed accumulation of fish feeds, including plant-based materials. Regarding the higher C/N ratios (9–17) in Unit II, these distinct properties in the sediments may indicate substantial inputs of fish feces, as well as feeding materials such as moist and dry pellets (Yokoyama et al. 2006). Given that terrestrial plant-based feeds are less digestible for carnivorous fish (De Silva and Anderson 1994), the higher elemental ratios in Unit II may also reflect the deposition of artificial feed pellets (C/N ratio > 7, Go et al. 2000). Therefore, the mixed accumulation of aquaculture-related OM, including unconsumed feeds, fish feces, and carcass, may occur depending on species metabolism and feed material composition (Yokoyama et al. 2006). In this context, we further investigated the isotopic compositions of bulk elements to provide insight into the source specificity of the mixed OM during aquaculture activities.

Generally, the $\delta^{13}C$ and $\delta^{15}N$ values of marine OM ranged from − 22 to − 18‰ and 3 to 12‰, respectively (Gao et al. 2012). The $\delta^{13}C$ values derived from terrestrial plants had wide isotopic ranges, that is, − 33 to − 21‰ for C_3 plants and − 17 to − 9‰ for C_4 plants (Pancost and Boot 2004; Lamb et al. 2006; Yu et al. 2010). The $\delta^{15}N$ values for terrestrial OM near aquaculture zones in South Korea were 7.4 ± 2.0‰ for C_3 plants and 10.7 ± 0.2‰ for C_4 plants (Kim et al. 2022). The isotopic compositions of fish feeds ($\delta^{13}C$: − 20.8 ± 0.2‰ and $\delta^{15}N$: 10 ± 0.0‰) and fecal wastes ($\delta^{13}C$: − 24.7 ± 0.4‰ and $\delta^{15}N$: 5.8 ± 0.5‰)

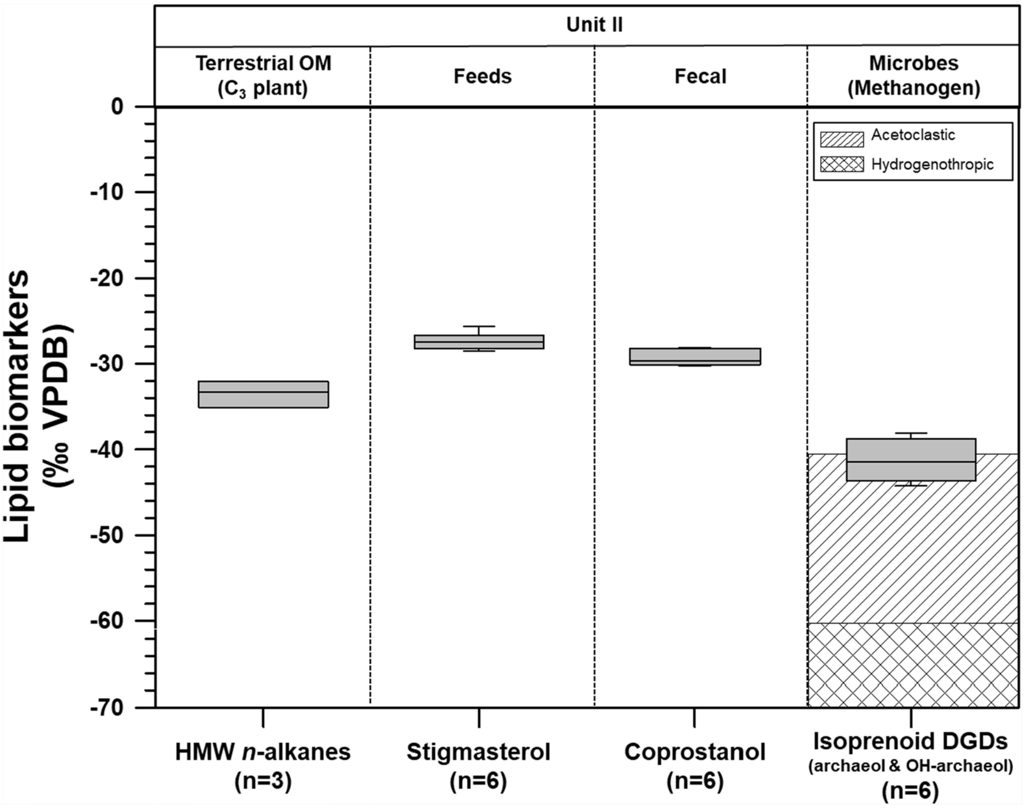

Fig. 6 Comparison of the carbon isotopic composition of selected lipid compounds, that is, HMW *n*-alkanes, stigmasterol, coprostanol, and isoprenoid DGDs for the Unit II section. The carbon isotope ranges of acetoclastic and hydrogenothropic methanogens are referred from the previous studies (Valentine et al. 2004; Londry et al. 2008)

have been often reported as differentiated isotopic properties compared to those of natural OM sources (Yokoyama et al. 2006). Considering these isotopic properties, the depleted isotopic patterns of both $\delta^{13}C_{TOC}$ and $\delta^{15}N_{TN}$ in Unit II (Fig. 3) likely resulted from the metabolic interaction between feed and feces. The $\Delta\delta^{13}C$ values (− 1.6 to 1.1‰) between feed and feces were likely derived from differentiated digestion of different feed materials (Ye et al. 1991; McGhie et al. 2000; Franco-Nava et al. 2004). The $\delta^{15}N$ values in feces may show more depleted patterns (2.7–5.4‰) than those of feed materials in line with excretion by fish species fed on artificial feed pellets (McGhie et al. 2000; Franco-Nava et al. 2004). Moisture feeds such as fish meal and raw fish are generally more easily digested by fish species (De Silva and Anderson 1994). Therefore, the depleted isotopic values of mixed OM in Unit II may partially reflect the substantial deposition of feces derived from effective digestion of feeds. In contrast, the overall isotopic patterns for Units I and III reflect the predominant terrestrial-derived OM, such as C_3 plants. Considering that allochthonous OM (> 90%) loading to Lake Soyang was concentrated during the summer monsoon (Kim et al. 2000), it is likely that these terrestrial sources during heavy rainfall may be intensively transported through surface runoff.

Lipid biomarkers such as *n*-alkanes and sterols provide more precise information on the origin of various OM in complex aquatic systems (Bourbonniere and Meyers 1996; Chikaraishi and Naraoka 2007; Derrien et al. 2017). LMW *n*-alkanes (C_{15} to C_{19}) are mainly derived from algae, bacteria, and submerged or floating aquatic macrophytes (Cranwell et al. 1987; Ficken et al. 2000; Pisani et al. 2013), while HMW *n*-alkanes (C_{27} to C_{35}) mainly originate from terrestrial higher plants (Ficken et al. 2000; van Dongen et al. 2008; Silva et al. 2012; Fang et al. 2014). Along with the bulk isotopic evidence, the increase in HMW *n*-alkanes in Unit III may reflect the enhanced input of terrestrial-derived OMs. Considering the transport of terrestrial OM (e.g., from agriculture and forests) as nonpoint sources, this may occur via surface runoff within Lake Soyang watershed (Derrien et al. 2018). The increased signatures of these lipids, which are resistant to degradation, likely indicate the significant accumulation of terrestrial sources within Unit III. In contrast, the vertical profiles of coprostanol, cholesterol, and stigmasterol showed higher concentrations in Unit

II compared to other units (Fig. 4), providing evidence for enhanced contributions of OM associated with aquaculture activities. First, 5β-stanols are commonly formed as reduction products of cholesterol and higher molecular weight congeners (e.g., campesterol, sitosterol, and stigmasterol) in the intestines of most higher mammals (Bull et al. 2002). Consequently, the presence of coprostanol, a product of the stepwise oxidation of the C-3 hydroxyl group in cholesterol, is considered a key indicator of substantial fecal inputs transported into natural systems (Leeming et al. 1996; McGhie et al. 2000; Martins et al. 2014; Derrien et al. 2017). However, reports of coprostanol production or its abundance in the fish feces are scarce. Notably, we detected coprostanol in the feces of common fish species (e.g., *Pagus major.*) collected at Korean coastal regions (Tongyeong, shown in Supplementary Information Fig. S3). The proportions of coprostanol and sitosterol in fish feces showed patterns similar to those observed in the feces of herbivores (e.g., cow and sheep; Bull et al. 2002). This suggests that fish may also produce coprostanol through microbial processes in their gut, similar to mammals. Based on this, we consider that the increased coprostanol level in Unit II is an indicator of fecal contamination resulting from past aquaculture activities. However, compared to the typical proxy for sewage pollution (coprostanol/(cholesterol + coprostanol), Grimalt et al. 1990), the range of 0.4 ± 0.1 observed in this study may be influenced by the differential loss of cholesterol and its preferential degradation under aerobic and/or anaerobic conditions. More research is needed to systematically confirm the biosynthetic process of these compounds across different trophic levels in aquaculture organisms. In addition to cholesterol, which originates from autochthonous sources and microbial reduction in natural environments (Volkman 1986; Bull et al. 2002; Jaffé et al. 2006), stigmasterol primarily originates from phytosterol found in plant tissues (Volkman 1986). While stigmasterol generally indicates the input of terrestrial plant material, the higher cholesterol/stigmasterol ratios (1.3–36.8) in Unit II likely reflect a significant contribution from animal consumers (cholesterol source), rather than a direct fingerprint of C_3 terrestrial plant mixed feed. Considering the ^{15}N-depleted TN in Unit II, which may reflect the predominant accumulation of fish feces fed on artificial feed, the detected stigmasterol abundance in Unit II likely represents the residual terrestrial plant-based mixed feeds, such as wheat flour, defatted soybean meal, and defatted rice bran meal. The degradation of aquaculture-related sterols occurs via aerobic bacteria in sedimentary environments (Bartlett 1987; Elhmmali et al. 1997), with further degradation possibly continuing under anaerobic conditions below the surface sediment (Canule and Martens 1996). However, given the enhanced steroid abundances in Unit II, which likely reflect the increased accumulation of aquaculture-derived OM, we infer that the preservation of these sterols may be sustained under anaerobic conditions at greater burial depths. Thus, the sedimentary OM sources in Unit II, derived from fish farming activities, may reflect a mixed contribution from both fish feces and uneaten dry feed.

4.2 Methanogenic signatures during past fish farming activities

Isoprenoid DGDs including archaeol and OH-archaeol, and isoprenoid GDGTs were detected in all Unit II sediments where aquaculture activities had occurred (Fig. 5). These lipids were also detected in Unit I and in some samples from Unit III (Fig. 5). Archaeol is ubiquitous in archaea, often being an indicator of methanogenic archaea in a wide range of environments including lakes (De Rosa and Gambacorta 1988; Koga et al. 1993, 1998; Pancost et al. 2011). OH-archaeol has been found in certain orders of methanogens such as *Methanosarcinales*, *Methanococcales*, *Methanopyrales*, *Thermoplasmatales*, *Sulfolobales*, and *Methanomicrobiales* (Kushwaha and Kates 1978; Koga et al. 1993, 1998; Koga and Morii 2005). Additionally, the predominance of ^{13}C-depleted OH-archaeol has also been observed in anaerobic methanotrophic archaeal groups, particularly ANME-2 and ANME-3, which utilize methane as their carbon sources (Blumenberg et al. 2004; Niemann and Elvert 2008). Given that isoprenoid DGDs may originate from various clades of methane-related archaeal communities mentioned above, identifying the source using diagnostic lipid compounds may face limitation in effectively distinguishing metabolic pathways between methanogens and methanotrophs. In fact, ANME-2d, found in freshwater environments, may potentially synthesize both archaeol and OH-archaeol (Kurth et al. 2019). However, the presence of methanotrophic archaeal communities in Lake Soyang sediments seems negligible due to the low sulfate concentrations (< 0.2 mM) (Jin et al. 1996), which limit the availability of electron acceptors necessary for methane oxidation under anoxic conditions. In such sulfate-limited conditions, most of the accumulated OMs may typically be converted by methanogens to methane. Methanogens are frequently dominant in Lake Soyang sediments (Go et al. 2000; Billard et al. 2015), and methane emission (ca. 69 g m^{-2} yr^{-1}) in this reservoir may be closely influenced by the increased accumulation of aquaculture-derived OM. Therefore, alongside variations in geochemical properties (e.g., porewater and gas components), further research is needed to precisely assess methanotrophic viability using RNA-based approaches (such as FISH or mRNA analysis) and to investigate substrate competitions between microbial communities (e.g., sulfate-reducing bacteria).

GDGT-0 is a predominant isoprenoid GDGT derived from methanogens (Schouten et al. 2013). Accordingly, GDGT-0 may have derived from methanogens preserved in core sediments of Lake Soyang. This hypothesis may be supported by GDGT-0/crenarchaeol ratios (> 0.8), which is regarded as a diagnostic index for methanogens (Blaga et al. 2009). The methane index has been used as an additional indicator for estimating the relative contribution between methane-related *Euryarchaeota* (represented by GDGT-1, GDGT-2, and GDGT-3) and benthic *Thaumarchaeota*, represented by crenarchaeol and its regioisomer (Zhang et al. 2011). This methane index showed little variation (~ 0.3) along with core depth (Fig. 5). In contrast to the dominance of GDGT-0 in core sediments, the overall low abundances of GDGT-1, GDGT-2, and GDGT-3, as well as crenarchaeol, may be related to the limited contributions from diverse methanogen species. Although the methane index has primarily been applied to marine deposits, particularly gas hydrate-bearing sediments, the low methane index values observed at our study core site may reflect the negligible contribution of ANME-1 archaea, which is known as representative methanotrophic communities. Previous studies have demonstrated the potential presence of other methane-related archaeal communities, including members of the orders *Methanomicrobiales* and *Methanosarcinales*, which belong to the phylum Euryarchaeota (Go et al. 2000). In contrast to the predominant abundance of GDGT-0, crenarchaeol may be less sensitive in tracing in situ aquatic archaeal input in lake environments compared to marine settings (Blaga et al. 2009). Therefore, lipid biomarker patterns, specifically, isoprenoid DGDs and GDGT-0, observed in Lake Soyang may indicate a strong methanogenesis process associated with early diagenesis within the aquaculture systems. Considering that the 16S rRNA sequences from the orders *Methanobacteriales and Methanococcales* were not recovered in Lake Soyang sediments (Go et al. 2000), further studies related to nucleic analysis are necessary for clarifying members of the domain methanogenic archaea.

In comparison with the $\delta^{13}C$ values of HMW *n*-alkanes (− 33.5 ± 1.5‰), stigmatosterol (− 27.4 ± 1.0‰), and coprostanol (− 29.3 ± 0.9‰), the $\delta^{13}C$ values of isoprenoid DGDs were more depleted (− 40.0 ± 3.2‰) in Units I and II (Fig. 6). Typically, *n*-alkanes are relatively depleted in ^{13}C, for the corresponding bulk tissue, because of biosynthetic fractions (Hayes 1993), with an isotopic offset ($\delta^{13}C_{bulk\text{-}lipid}$) of 5–10‰ for algae and 7–10‰ for vascular plants (Collister et al. 1994; Schouten et al. 1998; Wiesenberg et al. 2004). Based on the dominant abundance of HMW *n*-alkanes (C_{27}-C_{31}), their isotopic compositions may reflect the strong contribution of terrestrial vascular plants to the sediments of Lake Soyang. Considering that the $\delta^{13}C$ values of stigmasterol and coprostanol were similar to those of TOC, the increased abundance of these sterols may be involved in the mixed accumulation of feed materials and fish feces during aquaculture activities. Our isotopic results suggest that the increase in mixed by-products, such as residual feed and feces, can be identified using diagnostic sterols associated with aquaculture activities. Additionally, the more depleted isotopic signatures of isoprenoid DGDs may reflect contributions from different source organisms (Coolen et al. 2004; Naeher et al. 2014). If archaeal communities involved in anaerobic oxidation of methane were the source of specific biomarkers, that is, archaeol and OH-archaeol, these compounds would likely be highly depleted in ^{13}C (− 90 to − 133‰; Elvert et al. 1999; Hinrichs et al. 1999; Boetius et al. 2000; Pancost et al. 2000; Teske et al. 2002; Niemann and Elvert 2008). However, for the isotopic fractionation between OM and methanogenesis (Hinrichs et al. 2000; Niemann et al. 2005; Naeher et al. 2014), the key evidence as methanogenic sources came from the $\delta^{13}C$ values (− 30 to − 60‰) of methanogenic archaeal lipids (Hinrichs et al. 2000; Niemann et al. 2005). Therefore, isotopic values of isoprenoid DGDs may be indicative of the likely occurrence of methanogenesis in association with massive OM accumulation during past aquaculture activities in Lake Soyang.

4.3 Biogeochemical interaction between organic sources and methanogenesis

The PCA results showed variances in specific lipid abundances with distinct patterns between Unit III and Units I and II (Supplementary information Fig. S2). The dominance of sterols, particularly stigmasterol, and coprostanol, and isoprenoid DGDs may be related to the substantial accumulation of OM released during past aquaculture activities. This may have resulted in the occurrence of methanogenesis through sedimentary OM diagenesis (Naeher et al. 2014; Sabu et al. 2022). OM fermentation releases important metabolic substrates such as CO_2, H_2, organic acids, and inorganic nutrients, all of which are essential for methanogenic activity (Whiticar 1999). Characterized by $\varepsilon_{substrates-methane}$ values between − 35 and − 9‰ (Penning et al. 2006; Londry et al. 2008; Goevert and Conrad 2009), acetoclastic methanogenesis uses important precursors such as acetate and carbon dioxide for methane production. These isotopic variations can be distinguished from hydrogenotrophic methanogenesis, which exhibits a stronger fractionation of − 79 to − 28‰ (Valentine et al. 2004; Londry et al. 2008; Penger et al. 2012). Based on the distinguishing properties of the two types of methanogenesis, the ^{13}C-enriched isoprenoid DGDs observed in Lake Soyang suggest an acetoclastic methanogenic origin rather than a hydrogenotrophic or methanotrophic origin (Summons et al. 1994; Hinrichs et al. 2000; Blumenberg et al. 2004; Niemann et al. 2005, shown in Fig. 6). In fact, for Lake Soyang, archaeal sequences clustered with the order *Methanosarcinales* were closely related to *Methanosaeta* sp., which are exclusively acetoclastic methanogens (Koga et al. 1998; Go et al. 2000). Although clones of *Methanosaeta* species constituted a minority (27.5%), we infer that these acetoclastic communities may play a significant role in methane production under conditions of massive OM accumulation. This is particularly relevant given the high accumulation of feed residues in sediments, which are rich in polysaccharides (Dawood et al. 2018). These OM properties suggest that products derived from polysaccharide hydrolysis, such as glucose, fructose, and xylose, may be biodegraded into acetate, thereby stimulating acetoclastic methanogens (Rawoof et al. 2021). Considering that the by-products decomposed from aquatic-derived OM serve as primary substrates for methanogens under anaerobic conditions (Sabu et al. 2022), the massive accumulation of these OMs during past aquaculture activities likely promoted acetoclastic methanogenic processes. Meanwhile, for the order *Methanomicrobiales*, which accounted for 65% of all methanogens in Lake Soyang, the potential methanogenic activity of these communities has been shown to vary depending on the utilization of different substrates in laboratory experiments (acetate: 0.17 nM gdw^{-1} day^{-1} and formate: 0.42 nM gdw^{-1} day^{-1}, Go et al. 2000). This indicates that the methanogenic activities of the order *Methanomicrobiales* may depend on the availability of competitive substrates, in contrast to *Methanosaeta* sp., which can utilize non-competitive substrates (Muson et al. 1997). Given the predominant occurrence of acetoclastic methanogenesis in lake sediment systems (Mandic-Mulec et al. 2012), the contribution of these processes likely depends on environmental factors such as pH (Ye et al. 2012), temperature (Chen and Chang 2020), and the quantity and quality of OM in the sediments (Liu et al. 2012; Corbett et al. 2015; Hofmann et al. 2016). Although the metabolic pathways of acetoclastic and hydrogenotrophic methanogens remain incompletely understood through single-cell culture studies, the isotopic approach in this study provides a plausible alternative for distinguishing between different methanogenic pathways. From the perspective of active biogeochemical interactions, the significant increase in OM supply to lake sediment boundaries appears to enhance not only methanogen biomass but also distinct methanogenic processes within the sediment.

As aquaculture has recently emerged as the fastest growing food-producing sector to meet the increasing demand for aquatic products (FAO (Food and Agriculture Organization of the United Nations) 2020), negative issues, such as the increase in greenhouse gases derived from these activities, are continuously occurring (Sabu et al. 2022; Tan et al. 2023). In the case of Lake Soyang as a sedimentary methane reservoir, acetoclastic methanogensis involved in the decomposition of aquatic-derived OM, such as fish feeds and feces potentially, contributes to atmospheric methane emissions. Optimal supplies of feeds may be considered to better control and potentially reduce methane emissions in aquaculture systems. For instance, for the usage of proper feed types, lipid-rich feeds have a toxic effect on the activities of archaea, which may reduce methanogen abundance (Wang et al. 2017). Consider that methanogenesis may be inhibited by sulfate reduction and methane oxidation coupled with sulfate in anaerobic conditions (Ferry and Lessner 2008). The installation of fish farming system near coasts may be more effective for reducing methane emission at moderate sulfate levels. The predominant occurrence of methanogenesis would be determined by an early diagenetic process within sediment environments where high amounts of aquaculture-derived OM were massively deposited. Therefore, an effective way to reduce methanogenesis in lake systems would be to control aquaculture-derived residues with a properly estimated feed supply (Chik et al. 2017). After the aquaculture activities have been completed, the lake sediments should be plowed up, and the aquaculture-derived residues should be removed immediately to reduce the potential for methanogenesis.

5 Conclusion

The compound-specific isotopic analysis enabled the tracing of methane production from past fish farming activities. Corresponding with the increased bulk elements (i.e., TOC and TN) in Unit II, the impact of massive OM accumulation was observed. Along with distinct sterol compounds between each Unit section, these properties likely originated from mixed organic wastes, such as fish feed and feces. Under such conditions, isoprenoid DGDs, including archaeol and OH-archaeol, and GDGT-0 were dominant, mainly originating from methanogens involved in early diagenesis. Given the dominant occurrence of methanogens within Unit II, the isotopic compositions of these lipids suggest that organic materials derived from fish farming activities might have partially enhanced acetoclastic methanogenesis. These properties likely contributed to the increase in methane production resulting from the degradation of polysaccharide-rich feeds and feces. Thus, our findings provide key insights into the distributions and activities of methane-related microbes within a lake system subject to ongoing anthropogenic activities. Future work should also focus on the potential methane emissions at the water–atmosphere interface to better understand the feedback mechanisms involved in methane emissions within anthropogenically impacted lake systems, which serve as important methane reservoirs.

Abbreviations

TOC	Total organic carbon (TOC)
DGDs	Glycerol dialkyl diethers
GDGTs	Glycerol dialkyl glycerol tetraethers

Supplementary Information

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Supplementary material 1.

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Author contributions

Dong-Hun Lee wrote the initial draft of the manuscript. Jung-Hyun Kim and Kyung-Hoon Shin designed the isotope studies performed in Lake Soyang. Dong-Hun Lee, Sujin Kang, and Dahae Kim conducted the concentration and isotope analyses of bulk elements and lipid compounds. All the authors contributed to the manuscript drafts and manuscript finalization.

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Data availability

Data will be made available on request.

Declarations

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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