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Comparison of Modified Bligh-Dyer and Ultrasonic Organic Solvent Methods for GDGT Extraction from Surface Sediments of Lakes with Different Salinities

Rui Miao[1, 2], Ning Shi[1, 2], Zenghao Zhao[1], Hu Liu[3, 4], Xiangzhong Li[5], Huanye Wang[1]

[1]State Key Laboratory of Loess Science, Institute of Earth Environment, Chinese Academy of Sciences, Xi'an 710061, China

[2]University of Chinese Academy of Sciences, Beijing 100049, China.

3School of Water and Environment, Chang'an University, Xi'an 710064, China.

[4]Key Laboratory of Subsurface Hydrology and Ecological Effect in Arid Region of the Ministry of Education, Chang'an University, Xi'an 710064, China.

[5]Yunnan Key Laboratory of Earth System Science, School of Earth Science, Yunnan University, Kunming 650500, China.

Correspondence to: Huanye Wang (wanghy@ieecas.cn)

Abstract. Accurately quantifying core lipid (CL) and intact polar lipid (IPL) GDGTs is essential for investigating the sources of GDGTs and their responses to climatic and environmental changes in lacustrine systems. However, systematic comparisons of the performance of different methods for extracting GDGTs (both abundance and distribution) from lake sediments remain limited. In this study, we compared two ultrasonic organic solvent extraction methods, including a stepwise gradient extraction with dichloromethane/methanol (DCM/MeOH) solvent mixtures of different polarities and a single solvent extraction with DCM:MeOH (9:1, v:v), and two modified Bligh-Dyer (BD) methods (phosphate buffer; trichloroacetic acid) for extracting CL-GDGTs and IPL-GDGTs from saline and freshwater lake sediments. The results showed that, for CL-GDGTs, stepwise gradient extraction yielded the highest recovery, whereas no significant differences were observed in the CL-derived GDGTs proxies among the different extraction methods. For IPL-GDGTs, the BD (phosphate buffer) method achieved the highest recovery for isoprenoid GDGTs (isoGDGTs), while stepwise gradient extraction was most effective for extracting branched GDGTs (brGDGTs) and archaeol from saline lake sediments. Moreover, the consistently lower relative abundance of crenarchaeol to other isoGDGTs in CLs than in IPLs for all methods suggests that crenarchaeol is primarily produced in the lake water column, whereas other isoGDGTs have a relatively greater autochthonous production within the sediments or at the water-sediment interface. In saline lake sediments, we also observed higher relatively abundance of ≥7-methyl

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Preprint. Discussion started: 28 April 2026

brGDGTs and tetramethylated brGDGTs in IPLs than in CLs, indicating that their source bacteria are active at the water-sediment interface or in the sediments of saline lakes. These findings will provide insights for the quantitative analysis of GDGTs in lake sediments and for the study of their sources in lacustrine environments.

1 Introduction

Glycerol Dialkyl Glycerol Tetraethers (GDGTs) are unique lipid components of archaeal and bacterial cell membranes. Owing to their stable molecular structures and sensitive responses to environmental changes, they have become important biomarkers in the field of paleoclimate reconstruction (e.g., Hopmans et al., 2004; Weijers et al., 2007; Blaga et al., 2009; Turich and Freeman, 2011; Zhang et al., 2011; De Jonge et al., 2014; Wang et al., 2014; De Jonge et al., 2015; Wang et al., 2021). Environmental proxies based on GDGTs have been widely applied in paleoclimate and paleoenvironmental studies across various geological archives, including marine sediments, lakes, peat, and loess (e.g., Schouten et al., 2013; Inglis et al., 2022; Rezanka et al., 2023).

In living microbial cells, GDGTs primarily exist as intact polar lipids (IPL), whose molecular structure consists of a core tetraether skeleton with polar head groups attached at both ends. These polar head groups mainly include sugar-based head groups such as monohexose (MH), dihexose (DH), phosphohexose (PH), and hexose-phosphohexose (HPH) (Sturt et al., 2004; Schouten et al., 2008; Pitcher et al., 2011; Zheng et al., 2025), as well as phospholipid head groups (Sturt et al., 2004; Pitcher et al., 2009; Zheng et al., 2025). Following cell death, some polar head groups are removed through microbial degradation or during early diagenesis, gradually forming stable and well-preserved core lipid (CL) GDGTs. This process results in the common coexistence of both IPL-GDGTs and CL-GDGTs in natural samples.

Lake sediments serve as important geological archives for paleoenvironmental research. Although current paleoclimate reconstructions primarily rely on GDGT-based proxies derived from the distribution of CL-GDGTs, an increasing number of studies find that the production, transformation rates, and preservation efficiency of GDGTs significantly influence the accuracy of the resulting proxies. Therefore, investigating IPL-GDGTs in lakes is fundamental to understanding the response mechanisms of GDGTs

https://doi.org/10.5194/egusphere-2026-2188
Preprint. Discussion started: 28 April 2026

to environmental parameters. Previous studies have revealed that the production of IPL-GDGTs in the water column of both shallow and deep lakes exhibits pronounced seasonal variations (Buckles et al., 2014; Qian et al., 2019; van Bree et al., 2020; Baxter et al., 2021). Additionally, the primary productivity of GDGTs varies across different water depths, which can ultimately lead to biases in the "temperature signal" recorded in surface sediments (Weber et al., 2018; Meegan Kumar et al., 2019; Baxter et al., 2021). In addition to the water column, GDGTs can also be produced within lake surface sediments, introducing further biases into the proxies and thereby complicating the interpretation and application of these environmental proxies (Tierney et al., 2012; Qian et al., 2019; Raberg et al., 2022). Consequently, establishing an analytical method that enables the accurate quantification of both CL-GDGTs and IPL-GDGTs is essential for reducing quantification errors, improving data accuracy, distinguishing between living and sedimentary signals, and providing a more robust chemical foundation for paleoclimate reconstruction and microbial ecology research.

Currently, two major strategies are employed for the analysis of IPL-GDGTs. The first is the direct analysis method, which involves the direct determination of IPL-GDGTs in total lipid extracts (TLEs, including both CL and IPL fractions) using high-performance liquid chromatography coupled with electrospray ionization mass spectrometry (HPLC-ESI-MS) (e.g., Sturt et al., 2004; Huguet et al., 2010b; Van Mooy and Fredricks, 2010; Chen et al., 2016; Horai et al., 2019). The second is the indirect analysis method, which typically employs normal-phase high-performance liquid chromatography coupled with atmospheric pressure chemical ionization mass spectrometry (HPLC-APCI-MS) to measure CL-GDGTs derived from the acid hydrolysis of IPL-GDGTs, with the measured CL-GDGTs representing the IPL-GDGT concentration. The indirect method can be further divided into two approaches. One approach involves separating CL-GDGTs from IPL-GDGTs in TLEs using a silica gel column, followed by hydrolysis of the IPL-GDGT fraction and quantification of the resulting CL-GDGTs (e.g., Pitcher et al., 2009; Ingalls et al., 2012; Lengger et al., 2012; Zhu et al., 2013). The second approach employs a "subtraction method", in which the TLE is split into two aliquots, one aliquot is directly analyzed for its original CL-GDGT concentration, while the other aliquot is subjected to acid hydrolysis to convert IPL-GDGTs into CL-GDGTs, and the total CL-GDGTs (i.e., the sum of IPL-GDGTs and CL-GDGTs) is then measured. The IPL-GDGT concentration is calculated as the difference between the two measurements

https://doi.org/10.5194/egusphere-2026-2188
Preprint. Discussion started: 28 April 2026

(e.g., Huguet et al., 2010b; Lengger et al., 2012). Due to variations in the ionization efficiencies of different IPL-GDGTs in mass spectrometry, direct quantification of IPL-GDGTs is challenging (Zink et al., 2008). In contrast, the indirect method circumvents this difficulty by uniformly quantifying CL-GDGTs, and is therefore the most commonly employed approach (Bijl et al., 2025; Li et al., 2025).

For the extraction of GDGTs, various methods can be employed to extract TLEs from environmental or culture samples. These include ultrasonic organic solvent extraction (e.g., Schouten et al., 2007; Huguet et al., 2010b; Ingalls et al., 2012; Wang et al., 2017; Weber et al., 2017), the modified Bligh-Dyer (BD) method (e.g., Huguet et al., 2010b; Lengger et al., 2012; Buckles et al., 2014; Wang et al., 2017; Weber et al., 2017; Qian et al., 2019; van Bree et al., 2020; Raberg et al., 2022), Soxhlet extraction (e.g., Schouten et al., 2007; Huguet et al., 2010b; Lengger et al., 2012), accelerated solvent extraction (ASE) (e.g., Schouten et al., 2007; Huguet et al., 2010b; Lengger et al., 2012), and microwave-assisted extraction (MAE) (e.g., Escala et al., 2007; Huguet et al., 2010b; Pearson et al., 2011), among others. However, different extraction methods exhibit varying efficiencies for the extraction of CL-GDGTs and IPL-GDGTs (Bijl et al., 2025; Li et al., 2025). For CL-GDGTs, previous studies have shown that the GDGT-derived indices obtained using different extraction methods are consistent (Schouten et al., 2007; Wang et al., 2017); therefore, the choice of extraction method generally does not affect the application of CL-GDGT-based proxies. Nevertheless, several studies have demonstrated that extraction efficiencies for IPL-GDGTs vary significantly among different methods. Huguet et al. (2010b) compared the BD method, ultrasonication, Soxhlet extraction, ASE, and MAE, and found that Soxhlet extraction yielded the highest the highest recovery of total IPL-GDGTs from cultured samples and bay suspended particulate matter (SPM). Subsequently, however, Lengger et al. (2012) pointed out that different methods exhibit varying extraction efficiencies for GDGTs containing MH, DH, and HPH head groups; notably, the extraction efficiencies of Soxhlet extraction and ASE for HPH-crenarchaeol were significantly lower than that of the BD method, potentially due to the degradation of IPL-GDGTs under high-temperature conditions or adsorption of IPL-GDGTs to extraction vessels. Wang et al. (2017) observed that, for lake sediment and soil samples, ultrasonic solvent extraction recovered more CL-GDGTs than the BD method, whereas the BD method yielded more IPL-GDGTs. Weber et al. (2017) compared the efficiencies of ultrasonication and the modified BD method for extracting IPL-GDGTs

https://doi.org/10.5194/egusphere-2026-2188
Preprint. Discussion started: 28 April 2026

from lake filter samples, finding that although the BD method achieved higher extraction rates, substantial amounts of IPL-brGDGTs were lost in the aqueous phase (particularly in phosphate buffer) and in the solid residue. More recently, Evans et al. (2022) found that repeated freeze-thaw cycles prior to extraction facilitate cell membrane disruption, and that certain chemical detergents, such as cetyltrimethylammonium bromide (CTAB), can significantly improve IPL extraction efficiencies from archaeal pure cultures. In summary, for highly polar and thermally unstable IPL-GDGTs, extraction techniques involving high-temperature conditions may not be advisable. Although some emerging extraction methods offer high IPL-GDGT recoveries, they involve complex and time-consuming procedures that require further optimization or validation across a wide range of natural samples. Consequently, the BD method and ultrasonication remain the most widely used approaches for IPL-GDGT extraction (Bijl et al., 2025).

As noted above, existing comparative studies on IPL-GDGT extraction methods have predominantly focused on SPM from water columns or microbial culture samples, whereas systematic evaluations of IPL-GDGT (including both iso- and brGDGT) extraction from lake sediments remain limited. Given that sediments and SPM differ in matrix composition, particle structure, and lipid occurrence states, their extraction behaviors may also differ, potentially affecting the accuracy of quantitative results and subsequent environmental interpretations. In this study, we compared the performance of 4 methods for both extracting isoGDGTs and brGDGTs in surface sediments of 4 lakes (including both saline and freshwater lakes) on the Tibetan Plateau. The methods used for comparison include two modified Bligh-Dyer (BD) methods (phosphate buffer (P-buffer) and trichloroacetic acid (TCA)), stepwise gradient extraction with dichloromethane/methanol (DCM/MeOH) solvent mixtures of different polarities (MeOH, MeOH:DCM (1:1, v:v), and DCM), and single solvent extraction with MeOH:DCM (9:1, v:v). Our aim was to provide a basis for selecting appropriate analytical methods for studying CL-GDGTs and IPL-GDGTs for lake sediments. Furthermore, based on these comparisons, differences in distributions of CL-GDGTs and IPL-GDGTs were examined to elucidate potential sources of GDGTs in lacustrine systems.

https://doi.org/10.5194/egusphere-2026-2188
Preprint. Discussion started: 28 April 2026

2 Materials and methods

2.1 Study Area and Sample Collection

In this study, surface sediment samples were collected from four lakes in the northeastern Tibetan Plateau, including two saline lakes (Qinghai Lake and Sugan Lake) and two freshwater lakes (Keluke Lake and Erhai) (Fig. 1A). Qinghai Lake (36°32′–37°15′ N, 99°36′–100°47′ E) is the largest inland brackish lake in China, with a total dissolved solids (TDS) concentration of approximately 15 g/L, an average water depth of 17.9 m, and a lake water pH of approximately 9.1 (Wang and Dou, 1998). Sugan Lake (38°50′–38°54′ N, 93°45′–94°00′ E) is a sodium sulfate subtype brackish lake with a TDS concentration of approximately 32 g/L, an average water depth of 2.8 m, and a lake water pH of approximately 8.9 (Wang and Dou, 1998). Keluke Lake (37°14′–37°20′N, 96°51′–96°57′E) is a sodium sulfate subtype freshwater lake with a TDS concentration below 1 g/L, an average water depth of 2.9 m, and a lake water pH of approximately 8.0 (Wang and Dou, 1998). Erhai is a small freshwater lake formed after the water level decline of Qinghai Lake, located in the southeastern part of Qinghai Lake, with an average water depth of approximately 1.5 m, a pH of approximately 9.0, and a TDS concentration of approximately 1.2 g/L (Lanzhou Institute of Geology, Chinese Academy of Sciences et al., 1979).

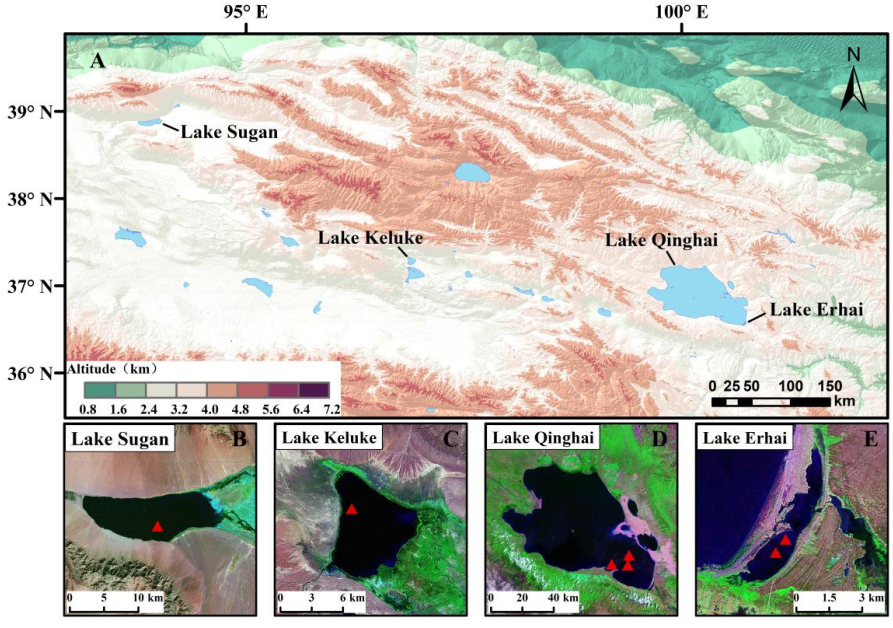

Figure 1. Location of the study lakes (A) and sampling sites in Sugan Lake (B), Keluke Lake (C), Qinghai Lake (D), and Erhai Lake (E)

https://doi.org/10.5194/egusphere-2026-2188
Preprint. Discussion started: 28 April 2026

Three surface sediment samples from Qinghai Lake were collected in 2021, with sampling site coordinates and corresponding water depths as follows: 100.432672 °E, 36.682831 °N (water depth: 23 m), 100.554561 °E, 36.739211 °N (water depth: 27 m), and 100.549753 °E, 36.682936 °N (water depth: 28 m) (Fig. 1D). Two surface sediment samples from Erhai were collected in 2019, with coordinates of 100.7216 °E, 36.55535 °N (water depth: 3 m) and 100.7267 °E, 36.56064 °N (water depth: 3 m) (Fig. 1E). The surface sediment sample from Keluke Lake was collected in 2023 at coordinates 96.873236 °E, 37.300164 °N, with a water depth of 6 m (Fig. 1C). The surface sediment sample from Sugan Lake was also collected in 2023 at coordinates 93.890912 °E, 38.854362 °N, with a water depth of 8 m (Fig. 1B).

2.2 Lipid extraction and fractionation

All sediment samples were freeze-dried and ground to homogeneity. The three samples from Qinghai Lake and the two samples from Erhai were each combined into one composite sample, resulting in four lake sediment samples (SG, QH, EH, and KLK). Each sample was divided equally into five aliquots. Among these, two aliquots were extracted using two modified Bligh-Dyer methods, two aliquots were extracted using two ultrasonic extraction methods, and the remaining aliquot was directly subjected to acid hydrolysis for the determination of total GDGTs (Fig. 2). All extractions were performed in polytetrafluoroethylene (PTFE) centrifuge tubes to minimize potential losses of IPL-GDGTs due to adsorption onto glass surfaces (Pitcher et al., 2009).

https://doi.org/10.5194/egusphere-2026-2188
Preprint. Discussion started: 28 April 2026

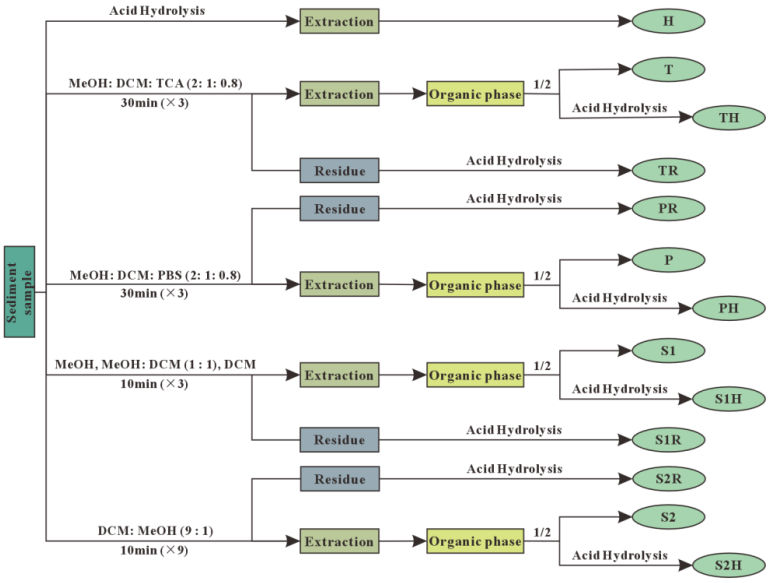

Figure 2. Flowchart of the pretreatment procedure for GDGT quantification

The modified BD methods were based on the original protocol described by Bligh and Dyer (1959), with the chloroform/methanol ($CHCl_3$/MeOH) system replaced by the less toxic DCM/MeOH system. P-buffer (White et al., 1979) and TCA aqueous solution (Nishihara and Koga, 1987) were used as the primary solvents for tetraether lipid extraction. Specifically, appropriate volumes of MeOH:DCM:0.1 M P-buffer (2:1:0.8, v:v:v; pH 7.4) and MeOH:DCM:5 % TCA (2:1:0.8, v:v:v) were added to two aliquots, respectively. Ultrasonic extraction was performed three times, each for 30 min, with an intermediate shaking step during each extraction. After each extraction, the mixture was centrifuged, and the supernatant was collected. The supernatants from the three extractions were combined. To the combined supernatant, additional DCM and P-buffer or TCA were added to adjust the final ratio to 1:1:0.9 (v:v:v) (MeOH:DCM:P-buffer/TCA). After thorough mixing, the mixture was allowed to separate into phases, and the DCM organic phase was collected. The aqueous phase was re-extracted twice with DCM. The organic phases from the three extractions were combined.

For the two aliquots subjected to ultrasonic extraction, different solvent mixtures of MeOH and DCM were used. One aliquot was subjected to three cycles of sequential extraction with MeOH, DCM:MeOH (1:1, v:v), and DCM, each extraction by ultrasonication for 10 min, for a total of 9 extractions. The other aliquot was extracted ultrasonically with DCM:MeOH (9:1, v:v) for nine cycles,

https://doi.org/10.5194/egusphere-2026-2188
Preprint. Discussion started: 28 April 2026

each lasting 10 min. The organic phases from the nine cycles were combined for each aliquot.

IPL-GDGTs were quantified indirectly using the "subtraction method", in which the removal of polar head groups via acid hydrolysis yields a difference in GDGT concentration that represents the IPL-GDGT concentration (Pitcher et al., 2009). Specifically, the combined organic phase from each extraction method was spiked with an appropriate amount of C_{46} GTGT internal standard and then divided into two equal portions. One portion was dried under N_2, redissolved in *n*-hexane, and filtered through a 0.22 μm syringe filter prior to analysis; the GDGTs measured in this portion correspond to CL-GDGTs. The other portion was dried under N_2 and hydrolyzed with 5% HCl in methanol at 70°C for 4 h. After cooling, an appropriate volume of DCM and H_2O was added to adjust the MeOH:DCM:H_2O ratio to 1:1:0.9 (v:v:v). The mixture was shaken and allowed to separate into phases, and the DCM organic phase was collected. The aqueous phase was re-extracted twice with DCM. The combined organic phases were washed three times with ultrapure water to remove residual acid, dried under N_2, redissolved in *n*-hexane, and filtered through a 0.22 μm PTFE filter prior to analysis; the GDGTs measured in this portion correspond to total extracted GDGTs. The IPL-GDGT concentration was calculated as the difference in GDGT concentration between the two portions.

In addition, the solid residues remaining after extraction were also subjected to acid hydrolysis to recover unextracted GDGTs (Huguet et al., 2010b; Tierney et al., 2012). Specifically, the solid residues were first treated with dilute hydrochloric acid (HCl) to remove carbonates, then freeze-dried again. Subsequently, 5 % HCl in MeOH was added, and the mixture was hydrolyzed at 70°C for 4 h. After cooling, the mixture was centrifuged, and the HCl/MeOH solution was collected. The solid residue was further extracted once each with DCM:MeOH (1:1, v:v) and DCM, respectively. The three extracts were combined. An appropriate volume of H_2O was added to adjust the MeOH:DCM:H_2O ratio to 1:1:0.9 (v:v:v). After thorough mixing and phase separation, the organic phase was collected, and the aqueous phase was re-extracted twice with DCM. The combined organic phases were washed three times with ultrapure water to remove residual acid, dried under N_2, filtered through a 0.22 μm PTFE filter, and analyzed.

2.3 Calculation of GDGT Proxies

The ACE salinity index, which is associated with archaeol and isoGDGTs, was calculated using the

https://doi.org/10.5194/egusphere-2026-2188
Preprint. Discussion started: 28 April 2026

formula defined by Turich and Freeman (2011) and modified by Wang et al. (2013).

$$ACE = \frac{archaeol}{archaeol + 10 \times GDGT\text{-}0} \times 100 \quad (1)$$

The primary indices related to isoGDGTs include: the GDGT-0/cren ratio based on the formula established by Blaga et al. (2009); the Methane Index (MI) calculated according to the formula defined by Zhang et al. (2011); and the relative proportion of crenarchaeol (%cren) based on the formula defined by Wang et al. (2014).

$$MI = \frac{GDGT\text{-}1 + GDGT\text{-}2 + GDGT\text{-}3}{GDGT\text{-}1 + GDGT\text{-}2 + GDGT\text{-}3 + crenarchaeol + crenarchaeol'} \quad (2)$$

$$\%cren = \frac{crenarchaeol}{GDGT\text{-}1 + GDGT\text{-}2 + GDGT\text{-}3 + crenarchaeol + crenarchaeol'} \quad (3)$$

The primary indices related to brGDGTs include: MBT' and MBT'_{5ME}, calculated using the formulas redefined by Peterse et al. (2014) and De Jonge et al. (2014) based on the original work of Weijers et al. (2007); the IR_{6ME} index, which represents the relative abundance of 6-methyl brGDGTs versus 5-methyl brGDGTs, calculated according to De Jonge et al. (2015); and the IR_{7ME} index, representing the relative abundance of 7-methyl brGDGTs, calculated according to Wang et al. (2021).

$$MBT' = \frac{Ia + Ib + Ic}{Ia + Ib + Ic + IIa + IIa' + IIb + IIb' + IIc + IIc' + IIIa + IIIa'} \quad (4)$$

$$MBT'_{5ME} = \frac{Ia + Ib + Ic}{Ia + Ib + Ic + IIa + IIb + IIc + IIIa} \quad (5)$$

$$IR_{6ME} = \frac{IIIa' + IIIb' + IIIc' + IIa' + IIb' + IIc'}{IIIa + IIIb + IIIc + IIa + IIb + IIc + IIIa' + IIIb' + IIIc' + IIa' + IIb' + IIc'} \quad (6)$$

$$IR_{7ME} = \frac{IIIa''' + IIa'''}{IIIa + IIIa' + IIIa''' + IIa + IIa' + IIa'''} \quad (7)$$

The BIT index, which involves both isoGDGTs and brGDGTs, was calculated according to Hopmans et al. (2004).

$$BIT = \frac{Ia + IIa + IIIa + IIa' + IIIa'}{Ia + IIa + IIIa + IIa' + IIIa' + crenarchaeol} \quad (8)$$

2.4 GDGT Analysis

GDGTs were analyzed using HPLC-Orbitrap Exploris 120. *n*-Hexane (A) and *n*-hexane:isopropanol

https://doi.org/10.5194/egusphere-2026-2188
Preprint. Discussion started: 28 April 2026

(9:1, v:v; B) were used as mobile phases. Isocratic elution was performed with 82 % A from 0 to 25 min, followed by a linear decrease to 65 % from 25 to 50 min, and then to 0 % from 50 to 80 min, which was maintained for 10 min, before finally returning to the initial conditions. The column temperature was maintained at 40°C, and the mobile phase flow rate was 0.2 mL/min. The evaporator temperature was set to 400°C, the ion transfer tube temperature was 300°C, and the spray current was 5 μA. The sheath gas flow rate was 30 Arb, and the auxiliary gas flow rate was 7 Arb. The resolution was set to 30,000 (FWHM). GDGTs and archaeol were analyzed in selected ion monitoring (SIM) mode targeting specific $[M+H]^+$ ions, with the following m/z values scanned: 1302.3227, 1300.3071, 1298.2914, 1296.2757, 1292.2444, 1050.041, 1048.0253, 1046.0097, 1036.0253, 1034.0097, 1031.994, 1022.0097, 1019.994, and 1017.9784. C_{46} GTGT was used as an internal standard for the quantification of GDGTs and archaeol (Huguet et al., 2006), assuming identical response factors.

3 Results

3.1 Extraction efficiencies of GDGTs for different pretreatment methods

Overall, the four methods yielded high recoveries of total GDGTs in lake sediments. The GDGT concentration obtained by direct acid hydrolysis was comparable to the sum of CL-GDGTs, IPL-GDGTs, and residual GDGTs for each method. The minimum recoveries of isoprenoid glycerol dialkyl glycerol tetraether (isoGDGTs), branched glycerol dialkyl glycerol tetraether (brGDGTs), and archaeol across different methods ranged from 63.8 % to 91.9 % (Fig. 3).

https://doi.org/10.5194/egusphere-2026-2188
Preprint. Discussion started: 28 April 2026

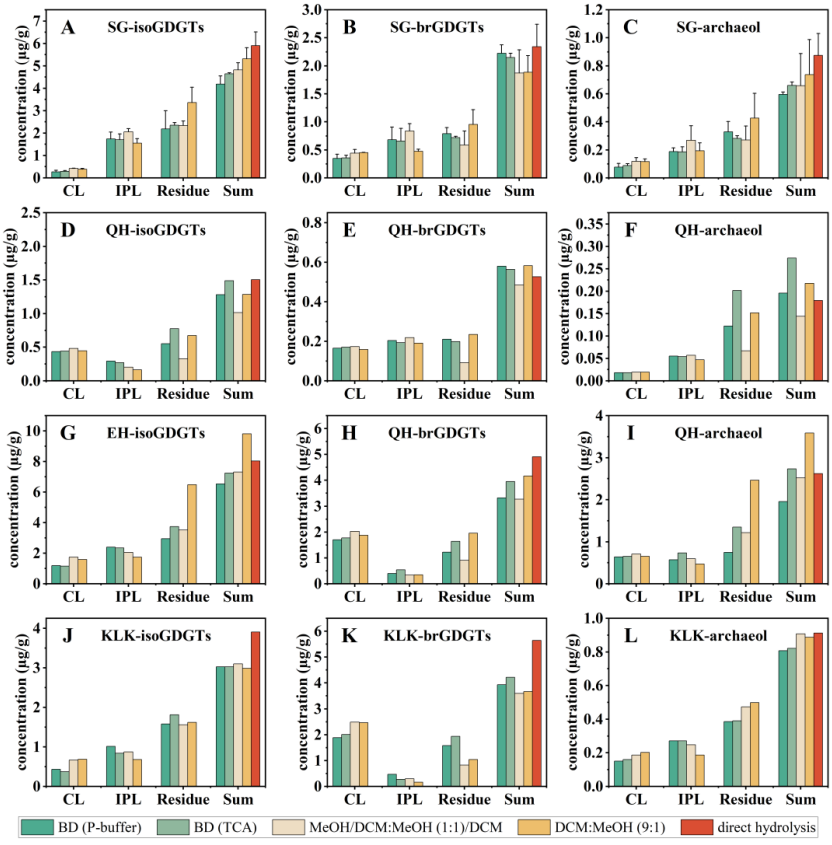

Figure 3. Differences in absolute abundances of CL-GDGTs, IPL-GDGTs, and residual GDGTs extracted by different methods for four samples (SG: A–C; QH: D–F; EH: G–I; KLK: J–L). "Sum" represents the total of CL-GDGTs, IPL-GDGTs, and residual GDGTs.

Abundant CL-GDGTs and IPL-GDGTs were detected in all four lake sediment samples. Compared with the two modified BD methods, the two ultrasonic extraction methods exhibited higher extraction efficiencies for CL-isoGDGTs, CL-brGDGTs, and CL-archaeol across all samples (Fig. 3), with this advantage being particularly pronounced in freshwater lake samples (EH and KLK). For the EH sample, the absolute abundances of CL-isoGDGTs and CL-brGDGTs extracted by the ultrasonic methods were approximately 500 ng/g and 200 ng/g higher, respectively, than those obtained by the BD methods (Fig. 3G, H); for the KLK sample, the corresponding differences were approximately 300 ng/g and 500 ng/g, respectively (Fig. 3J, K). However, the extraction efficiencies for IPL-GDGTs varied significantly among the different methods across samples (Fig. 3). Specifically, for the SG sample, stepwise gradient extraction with MeOH, DCM:MeOH (1:1, v:v), and DCM yielded the highest extraction efficiencies for IPL-isoGDGTs, IPL-brGDGTs, and IPL-archaeol (Fig. 3A–C). In contrast, the BD (P-buffer) method achieved the highest extraction efficiencies for IPL-isoGDGTs, IPL-brGDGTs, and IPL-archaeol in the KLK sample (Fig. 3J–L). For the QH sample, the BD (P-buffer) method was most effective for extracting IPL-isoGDGTs, while the other three methods showed comparable extraction efficiencies for IPL-

https://doi.org/10.5194/egusphere-2026-2188
Preprint. Discussion started: 28 April 2026

brGDGTs and IPL-archaeol (Fig. 3D–F). For the EH sample, the BD (P-buffer) method exhibited the highest extraction efficiency for IPL-isoGDGTs, whereas the BD (TCA) method performed best for IPL-brGDGTs and IPL-archaeol (Fig. 3G–I). Notably, across all samples, ultrasonic extraction with DCM:MeOH (9:1, v:v) yielded significantly lower extraction efficiencies for IPL-GDGTs compared to the other three methods (Fig. 3).

To eliminate the influence of heterogeneity in total GDGT concentration among samples, the total GDGTs (Sum) obtained from each pretreatment method were normalized to 100 %, and the relative proportions of CL-GDGTs, IPL-GDGTs, and residual GDGTs were subsequently calculated. For the SG sample, the relative proportions of IPL-isoGDGTs, IPL-brGDGTs, and IPL-archaeol were all higher than those of their corresponding CL counterparts. Under the optimal extraction method, CL-isoGDGTs and IPL-isoGDGTs accounted for approximately 8.8 (±0.3) % and 42.7 (±1.3) %, respectively; CL-brGDGTs and IPL-brGDGTs accounted for approximately 24.4 (±3.4) % and 45.3 (±4.3) %, respectively; and CL-archaeol and IPL-archaeol accounted for approximately 18.3 (±2) % and 40.6 (±2) %, respectively (Fig. 4A–C). For the QH sample, the relative proportion of IPL-isoGDGTs was lower than that of CL-isoGDGTs (12.7 % and 47.4 %, respectively, under the optimal extraction method), while IPL-brGDGTs and CL-brGDGTs exhibited similar proportions (45.2 % and 35.7 %, respectively, under the optimal extraction method). In contrast, the proportion of IPL-archaeol was higher than that of CL-archaeol (39.8 % and 13.4 %, respectively, under the optimal extraction method) (Fig. 4D–F). In the two freshwater lake samples (EH and KLK), the proportions of IPL-isoGDGTs and IPL-archaeol were higher than those of their corresponding CL components, whereas the proportion of IPL-brGDGTs was markedly lower than that of CL-brGDGTs. For the EH sample under the optimal extraction method, the relative proportions of IPL-isoGDGTs and CL-isoGDGTs were 23.8 % and 36.6 %, respectively; IPL-brGDGTs and CL-brGDGTs accounted for 62 % and 13.6 %, respectively; and IPL-archaeol and CL-archaeol accounted for 32.8 % and 29.2 %, respectively (Fig. 4G–I). For the KLK sample under the optimal extraction method, the relative proportions of IPL-isoGDGTs and CL-isoGDGTs were 23 % and 33.4 %, respectively; IPL-brGDGTs and CL-brGDGTs accounted for 69.1 % and 11.9 %, respectively; and IPL-archaeol and CL-archaeol accounted for 22.8 % and 33.7 %, respectively (Fig. 4J–L).

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Preprint. Discussion started: 28 April 2026

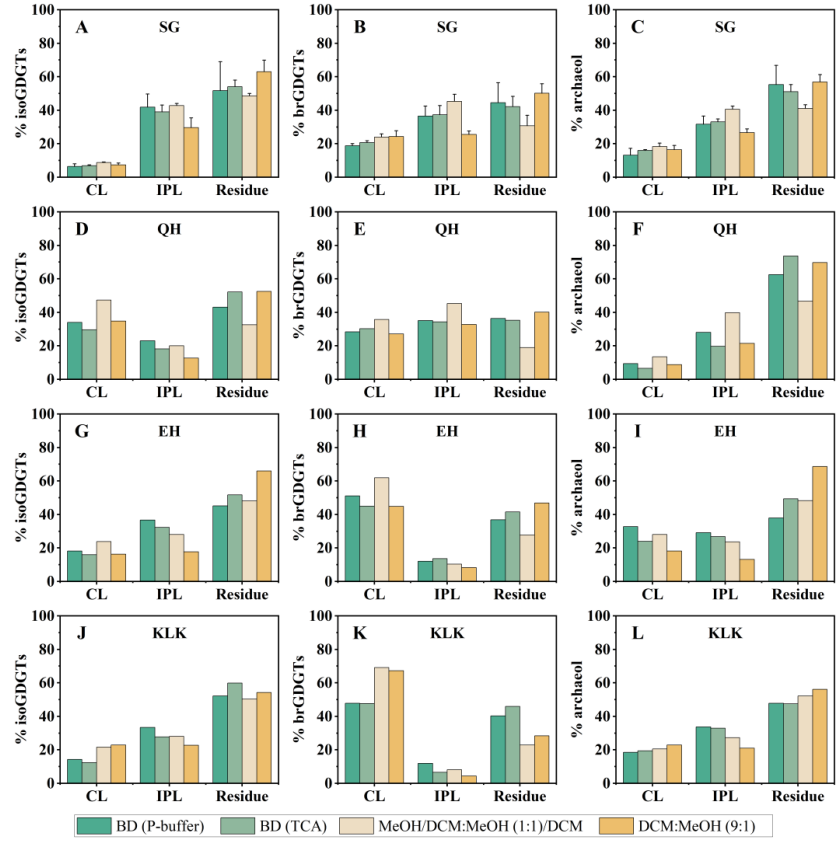

Figure 4. Differences in the yield of CL-GDGTs, IPL-GDGTs, and residual GDGTs extracted by different methods for four samples (SG: A–C; QH: D–F; EH: G–I; KLK: J–L).

Furthermore, the proportion of GDGTs released by hydrolysis of the residues was relatively high across all methods. For the SG sample, the DCM:MeOH (9:1, v:v) ultrasonic extraction method yielded the highest residual GDGT proportions for isoGDGTs, brGDGTs, and archaeol, accounting for 63 (±6.9) %, 50.1 (±5.6) %, and 56.9 (±4.3) %, respectively (Fig. 4A–C). In contrast, stepwise gradient ultrasonic extraction with MeOH, DCM:MeOH (1:1, v:v), and DCM resulted in the lowest residual GDGT proportions for these three components, at 48.5 (±1.6) %, 30.8 (±6.2) %, and 41.1 (±2.2) %, respectively (Fig. 4A–C). For the QH sample, the DCM:MeOH (9:1, v:v) ultrasonic extraction method yielded the highest residual GDGT proportions for isoGDGTs and brGDGTs, at 52.6 % and 40.2 %, respectively (Fig. 4D, F), while the BD (TCA) method yielded the highest residual proportion for archaeol at 73.7 % (Fig. 4F). The stepwise gradient ultrasonic extraction with MeOH, DCM:MeOH (1:1, v:v), and DCM yielded the lowest residual proportions for all three components, at 32.6 %, 19 %, and 46.7 %, respectively (Fig. 4D–F). For the EH sample, the DCM:MeOH (9:1, v:v) ultrasonic extraction method yielded the highest residual GDGT proportions for isoGDGTs, brGDGTs, and archaeol, at 66 %, 46.9 %, and 68.7 %, respectively (Fig. 4G–I). The BD (P-buffer) method yielded the lowest residual

https://doi.org/10.5194/egusphere-2026-2188
Preprint. Discussion started: 28 April 2026

proportions for isoGDGTs and archaeol, at 45.2 % and 38 %, respectively (Fig. 4G, I), while the stepwise gradient ultrasonic extraction with MeOH, DCM:MeOH (1:1, v:v), and DCM yielded the lowest residual proportion for brGDGTs at 27.8 % (Fig. 4H). For the KLK sample, the BD (TCA) method yielded the highest residual GDGT proportions for isoGDGTs and brGDGTs, at 59.9 % and 45.9 %, respectively (Fig. 4J, K), while the DCM:MeOH (9:1, v:v) method yielded the highest residual proportion for archaeol at 56.2 % (Fig. 4L). The stepwise gradient ultrasonic extraction with MeOH, DCM:MeOH (1:1, v:v), and DCM yielded the lowest residual proportions for isoGDGTs and brGDGTs, at 50.3 % and 22.8 %, respectively (Fig. 4J, K), whereas the BD (TCA) method yielded the lowest residual proportion for archaeol at 47.6 % (Fig. 4L).

3.2 Distributions of CL-GDGTs and IPL-GDGTs using different extraction methods

In three replicate experiments of the SG sample using different extraction methods, the standard errors for MBT', MBT'$_{5ME}$, IR$_{6ME}$, IR$_{7ME}$, MI, and BIT in the CL-GDGT fraction did not exceed 0.01 (Fig. 5A–E, G). The maximum standard error for %cren was 0.04 (Fig. 5H), for ACE was 0.68 (Fig. 5I), and for GDGT-0/cren was 5.4 (Fig. 5F). For the IPL-GDGT fraction in the three replicate experiments of the SG sample, the maximum standard error for MBT'$_{5ME}$ was 0.01 (Fig. 5B); for MBT', IR$_{6ME}$, IR$_{7ME}$, and BIT, the maximum standard error was 0.03 (Fig. 5A, C–E); for MI and %cren, the maximum standard error was 0.06 (Fig. 5G, H); for ACE, the maximum standard error was 0.39 (Fig. 5I); while the standard error for GDGT-0/cren was relatively large, with a maximum of 109.16 (Fig. 5F). Therefore, the systematic errors introduced by the experimental process and instrument response were minimal and negligible when comparing differences among extraction methods in this study, and replicate analysis were not performed on the other samples.

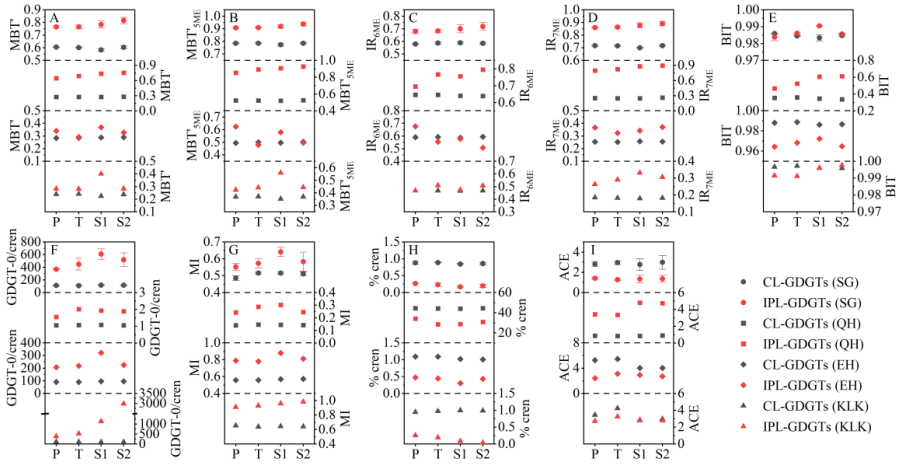

Figure 5. Differences in the major indices of CL-GDGTs and IPL-GDGTs obtained using different extraction methods. P: BD (P-buffer) method; T: BD (TCA) method; S1: stepwise gradient extraction with MeOH, DCM:MeOH (1:1, v:v), and DCM; S2: DCM:MeOH (9:1, v:v).

The distributions of the CL-GDGT fraction did not show significant differences among the four extraction methods, as indicated by multiple GDGT-based indices. For the four lake sediment samples,

https://doi.org/10.5194/egusphere-2026-2188
Preprint. Discussion started: 28 April 2026

the standard deviations of MBT', MBT'_{5ME}, IR_{6ME}, IR_{7ME}, MI, and BIT under the four extraction methods were all below 0.01 (Fig. 5A–E, G); the standard deviation of GDGT-0/cren was below 3.85 (Fig. 5F); the standard deviation of %cren was below 0.23 (Fig. 5H); and the standard deviation of ACE was below 0.78 (Fig. 5I). In contrast, the major proxies of the IPL-GDGT fraction exhibited more pronounced differences among the four extraction methods. For the four lake sediment samples, the standard deviations of MBT', MBT'_{5ME}, IR_{6ME}, IR_{7ME}, MI, and BIT under the four extraction methods were all below 0.07 (Fig. 5A–E, G); the standard deviation of %cren was below 2.59 (Fig. 5H); and the standard deviation of ACE was below 0.79 (Fig. 5I). For the SG, QH, and EH samples, the standard deviations of GDGT-0/cren under the four extraction methods were all below 52.62, whereas for the KLK sample, the GDGT-0/cren value obtained using the DCM:MeOH (9:1, v:v) extraction method was anomalously high, differing significantly from the results of the other methods (Fig. 5F).

Notable differences were observed between the IPL-GDGT and CL-GDGT fractions for various proxies. Under different extraction methods, the MBT', MBT'_{5ME}, IR_{6ME}, and IR_{7ME} values of the IPL-GDGT fraction were higher than those of the corresponding CL-GDGT fraction in the two saline lake samples (SG and QH), whereas this pattern was not evident in the two freshwater lake samples (EH and KLK) (Fig. 5A–D). For the SG sample, the MBT', MBT'_{5ME}, IR_{6ME}, and IR_{7ME} values of the IPL-GDGT fraction were approximately 0.18, 0.14, 0.12, and 0.16 higher, respectively, than those of the CL-GDGT fraction across the four methods (Fig. 5A–D); for the QH sample, the corresponding differences were approximately 0.43, 0.38, 0.11, and 0.60, respectively (Fig. 5A–D). Across all samples, the GDGT-0/cren and MI values of the IPL-GDGT fraction obtained by different methods were higher than those of the corresponding CL-GDGT fraction, while the %cren values were lower than those of the CL-GDGT fraction (Fig. 5F–H). The BIT and ACE indices exhibited no clear patterns between the IPL-GDGT and CL-GDGT fractions or among different lakes (Fig. 5E, I).

4 Discussion

4.1 Differences in recovery of CL-GDGTs and IPL-GDGTs by different methods

Regardless of whether assessed by absolute abundance or relative percentage, ultrasonic extraction methods generally exhibited higher efficiencies for extracting CL-GDGTs from both freshwater and saline lake sediments compared to the BD methods (Figs. 3 and 4), particularly for CL-isoGDGTs and CL-brGDGTs in freshwater lake samples (Fig. 3G, H, J, K). Similar results have been observed in previous comparative studies on GDGT extraction methods (Huguet et al., 2010b; Wang et al., 2017). However, Weber et al. (2017) previously compared GDGT extraction from lake filter samples and found no significant differences between the BD and ultrasonic extraction methods for CL-GDGTs. This suggests that the observed extraction differences may arise not only from the affinity of GDGTs for the extraction solvents but also from the environmental matrix to which GDGTs are associated or from differences in their potential biological sources.

Notably, the extraction of IPL-GDGTs is more complex than that of CL-GDGTs. Among the solvents used, MeOH:DCM (9:1) consistently yielded the lowest extraction efficiencies for IPL-GDGTs across all samples (Fig. 4). For IPL-isoGDGTs in all four lake sediments, as well as for IPL-brGDGTs

https://doi.org/10.5194/egusphere-2026-2188
Preprint. Discussion started: 28 April 2026

and IPL-archaeol in the two freshwater lake sediments, the BD methods achieved the highest extraction efficiencies. However, in the two saline lake sediments, stepwise gradient ultrasonic extraction with MeOH, DCM:MeOH (1:1, v:v), and DCM outperformed the BD methods in extracting IPL-brGDGTs and IPL-archaeol (Fig. 3B, C, E, F). Currently, knowledge regarding the biological sources and IPL composition differences of living GDGTs between freshwater and saline lakes remains limited. Nonetheless, notable differences exist in the composition of IPL-brGDGTs between saline and freshwater lake sediments. In saline lake samples (SG and QH), the IR_{7ME} index, which represents the relative abundance of ≥7-methyl brGDGTs (IIIa''', IIa'''') in polar lipids, was substantially higher than that in freshwater lake samples (EH and KLK) (Fig. 5D). This compositional difference has also been reported in global lake studies and may reflect that the producer communities of heptamethyl brGDGTs in saline lakes are significantly regulated by salinity, analogous to the influence of salinity on the community composition of Euryarchaeota and other archaea (Turich and Freeman, 2011; He et al., 2020; Wang et al., 2021). These brGDGTs and archaeol with specific polar head groups may be more readily extracted by the MeOH/DCM:MeOH (1:1)/DCM solvent combination, which may partly explain the differences in IPL-brGDGT extraction efficiencies between saline and freshwater lake samples observed across methods.

Previous comparisons of pretreatment methods using culture samples or lake filter samples revealed that the BD method exhibited low recovery of highly polar IPL-GDGTs from the aqueous phase into the MeOH/DCM mixed organic phase (Huguet et al., 2010b; Weber et al., 2017), with the BD (P-buffer) method resulting in up to 75 % loss of IPL-brGDGTs (Weber et al., 2017). However, in the four lake sediment samples of this study, the differences in brGDGT recovery between the BD methods (CL+IPL+Residue) or ultrasonic extraction methods (CL+IPL+Residue) and direct acid hydrolysis were not significant. Moreover, the normalized percentage abundances of each GDGT fraction were consistent with the absolute abundances (Figs. 3 and 4). This indicates that GDGT loss in the aqueous phase was minimal in this study, much lower than 75 %. This discrepancy may stem from the much higher abundances of IPL-GDGTs representing living biomass in culture samples and SPM compared to sediment samples, and may also relate to differences in the polar head groups of GDGTs within their respective matrices. Although the exact reasons remain unclear, it is noteworthy that when performing quantitative analysis of IPL-GDGTs, attention should be paid to the differential extraction efficiencies of BD methods for different types or hydrophilic/hydrophobic IPL-GDGTs to avoid losing important GDGT compositional information.

Compared to the potential loss of GDGTs in the aqueous phase, acid hydrolysis of the post-extraction solid residues revealed that substantial amounts of GDGTs remained in the solid phase, with abundances even exceeding those of CL-GDGTs or IPL-GDGTs (Figs. 3 and 4). Such GDGTs requiring hydrolysis for release have been reported in lake sediment and soil samples; for instance, the proportion of conventionally unextractable isoGDGTs in soil residues can reach up to 90 % (Tierney et al., 2012). Similarly, Huguet et al. (2010b) noted that the majority of lipids in exponentially growing cells were not effectively recovered by the tested methods, suggesting that these lipids may not originate from actively dividing cells in the environment, and that approximately 70 % of IPL-GDGTs remained associated with cellular material in some form, releasable only through acid hydrolysis. Furthermore, distinct differences

https://doi.org/10.5194/egusphere-2026-2188
Preprint. Discussion started: 28 April 2026

were observed among the extraction methods. Weber et al. (2017) reported for filter samples that only the ultrasonic method resulted in significant loss of GDGTs to the solid residue, whereas the BD method exhibited minimal solid-phase loss. In contrast, for the sediment samples in this study, both the BD and ultrasonic methods resulted in relatively high proportions of GDGTs in the residues, accounting for approximately 20 %–70 % of total GDGTs (Fig. 4), with the stepwise gradient method using MeOH, DCM:MeOH (1:1, v:v), and DCM yielding the lowest residual GDGT amounts. Previous studies have suggested that CL-GDGTs or IPL-GDGTs may not be released by conventional solvent extraction due to adsorption onto soil or sediment macromolecules or mineral matrices (Tierney et al., 2012; Pei et al., 2025), or because certain intact microbial cells possess "inherent recalcitrance" to organic solvent extraction (Huguet et al., 2010b; Sinninghe Damsté et al., 2011, 2014; Cario et al., 2015). A recent study by Pei et al. (2025) found that high-temperature pyrolysis could effectively enhance the release of bound CL-GDGTs from minerals, but further extraction of IPL-GDGTs was not conducted, as high temperatures may potentially affect the stability of IPL-GDGTs (Lengger et al., 2012). Therefore, further attention should be directed toward the extraction methods and composition of bound IPL-GDGTs.

4.2 Differences in CL-GDGT and IPL-GDGT indices under different methods

Although certain differences exist in the absolute abundances of CL-GDGTs obtained by different extraction methods, the indices derived from CL-isoGDGTs, CL-brGDGTs, and CL-archaeol show strong consistency across methods (Fig. 5), indicating that the extraction efficiencies for different CL-GDGT structures vary minimally among methods. This finding is consistent with previous method comparison studies conducted on lake sediments, marine sediments, and peat, where no significant differences in GDGT-based proxies were observed among extraction methods (Schouten et al., 2007; Huguet et al., 2010a; Zhang et al., 2012; Wang et al., 2017). Therefore, the choice of extraction method does not affect the application of CL-GDGT-based proxies in paleoclimate reconstruction and related studies.

In contrast to CL-GDGT proxies, some IPL-GDGT-related proxies exhibit considerable variability across different extraction methods. For example, the MBT'$_{5ME}$ index for the EH sample obtained by the BD (P-buffer) method, and the MBT' and MBT'$_{5ME}$ indices for the KLK sample obtained by the stepwise gradient method using MeOH, DCM:MeOH (1:1), and DCM, are approximately 0.1 higher than those obtained by other methods. For indices such as GDGT-0/cren, where the numerator and denominator are independent variables with small covariance, the differences are further amplified. For the SG sample, the GDGT-0/cren index differs by up to 250 among methods, accompanied by high standard deviations among replicates. For the KLK sample, the GDGT-0/cren value obtained using the DCM:MeOH (9:1) method is several times higher than those obtained by other methods.

Two possible reasons may account for these observations. On one hand, the polarity of different polar head groups varies considerably, leading to differences in their extractability; consequently, the varying polarities of extraction solvents result in non-proportional extraction of different head group types. On the other hand, when GDGT absolute abundances are low, as is the case for IPL-brGDGTs in freshwater lakes, the variability in calculated proxy indices is further amplified. Therefore, for samples with relatively low IPL-GDGT concentrations, caution should be exercised when applying the

https://doi.org/10.5194/egusphere-2026-2188
Preprint. Discussion started: 28 April 2026

subtraction method to calculate IPL-GDGT-based proxies.

4.3 Implications for production of GDGTs

Based on variations among different isoGDGT producers, several indices have been developed to trace the contributions of distinct isoGDGT producers in aquatic environments, including GDGT-0/crenarchaeol (Blaga et al., 2009), MI (Zhang et al., 2011), and %cren (Wang et al., 2014). Differences in these indices across various environmental archives can be used to reconstruct changes in isoGDGT producer communities. Multiple modern lake process studies have indicated that high GDGT-0/crenarchaeol ratios or high GDGT-0 abundances are commonly observed in anoxic bottom waters, suggesting that GDGT-0 is predominantly produced under anoxic conditions (Blaga et al., 2009; Baxter et al., 2021). However, GDGT-0 is also abundant in anoxic sediments, with its concentration often increasing with sediment depth (Tierney et al., 2012). Although the behavior of IPL-GDGTs during transport and degradation from the water column to sediments is difficult to constrain precisely, the higher GDGT-0 concentration and GDGT-0/crenarchaeol ratios in sediments compared to SPM appear to support in situ methanogenic activity within the sediments (Blaga et al., 2009; Sinninghe Damsté et al., 2009; Tierney et al., 2012; Lengger et al., 2014). In this study, the GDGT-0/cren and MI values in the IPL-GDGT fraction of the four lake sediments were significantly higher than those in the CL-GDGT fraction, whereas %cren in the IPL-GDGT fraction was lower than that in the CL-GDGT fraction, regardless of the extracting method used (Fig. 5E–H). These findings suggest that biologically active crenarchaeol is primarily produced in the lake water column rather than being newly formed in the sediments, whereas GDGT-0, GDGT-1, GDGT-2, and GDGT-3 may undergo substantial in situ production within the sediments or at the water-sediment interface.

Although the in-situ production of brGDGTs in lake water columns and sediments has been widely documented (e.g., Tierney et al., 2012; Buckles et al., 2014), little attention has been paid to the in-situ production of more higher methyl positional isomers of brGDGTs (IIIa''', IIa'''), which are predominantly abundant in saline lakes (Wang et al., 2021; Kou et al., 2022). In this study, the IR_{7ME} index derived from IPL-GDGTs was significantly higher than that derived from CL-GDGTs across the four lake sediments (Fig. 5D), indicating the substantial presence of living or recently produced ≥7-methyl brGDGTs in lake sediments. Although the contributions of ≥7-methyl brGDGTs produced in sediments or bottom waters remain unclear, the difference in IR_{7ME} between the IPL-GDGT and CL-GDGT fractions was markedly larger than those observed for MBT', MBT'_{5ME}, and IR_{6ME} (Fig. 5A–D). This suggests that ≥7-methyl brGDGTs may be produced in larger quantities compared with other brGDGTs within sediments (or at the water-sediment interface), or alternatively, they may be generated in deeper water layers and degrade more slowly compared to other brGDGTs. Furthermore, in the two saline lake sediment samples, the MBT', MBT'_{5ME}, IR_{6ME}, and IR_{7ME} indices derived from IPL-GDGTs were consistently higher than those derived from CL-GDGTs (Fig. 5A–D). This may imply that bacteria producing 7-methyl brGDGTs may also synthesize other types of brGDGTs. A previous global lake survey by Wang et al. (2021) indicated that the relative abundance of 7-methyl brGDGTs is strongly regulated by salinity, and that MBT'_{5ME} is influenced by salinity-driven isomerization of brGDGTs. This is consistent with the observations in this study, where producers of 7-methyl brGDGTs may simultaneously produce tetramethylated brGDGTs

https://doi.org/10.5194/egusphere-2026-2188
Preprint. Discussion started: 28 April 2026

(Ia, Ib and Ic), leading to coordinated variations in the MBT' and MBT'$_{5ME}$ indices and consequently introducing bias into temperature proxies.

5 Conclusions

In this study, by comparing the performance of two modified Bligh-Dyer methods and two ultrasonic extraction methods for GDGT extraction in sediment samples from two saline lakes and two freshwater lakes, we found that stepwise gradient ultrasonic extraction with MeOH, DCM:MeOH (1:1, v:v), and DCM generally yielded the highest extraction efficiency for CL-GDGTs. However, the major indices derived from CL-GDGTs showed no significant differences among the different extraction methods, indicating that extraction methods do not affect the calculation of these proxies in paleoclimate reconstruction. In contrast, the extraction results for IPL-GDGTs varied considerably, with differences observed across methods, lake salinity regimes (saline vs. freshwater), and GDGT components (isoGDGTs, brGDGTs, and archaeol). Therefore, when quantifying IPL-GDGTs, it is recommended to select an appropriate extraction method through preliminary comparative experiments. Furthermore, a substantial proportion of GDGTs may be occluded within sediments and cannot be fully extracted by conventional solvents.

Comparisons of distributional differences between CL-GDGT and IPL-GDGT fractions further revealed that crenarchaeol is primarily produced in the lake water column, whereas GDGT-0, GDGT-1, GDGT-2, and GDGT-3 undergo significant in-situ production in sediments or at the water-sediment interface. In saline environments, ≥7-methyl brGDGTs might also be abundantly produced in sediments or at the water-sediment interface, and their source bacteria are likely to also synthesize tetramethylated brGDGTs, thereby affecting the interpretation and application of the relevant methylation indices.

Data availability

The data used for this study can be found in the supplement.

Author contributions

HW conceptualized the study, supervised the project and secured funding; RM conducted the experiments, analyzed the data and wrote the manuscript draft; RM, NS, ZZ, HL, and XL developed the methodology; HW reviewed and edited the manuscript.

https://doi.org/10.5194/egusphere-2026-2188
Preprint. Discussion started: 28 April 2026

Competing interests

The contact author has declared that none of the authors has any competing interests.

Disclaimer

The authors declare that this work has not been published nor is under consideration anywhere else.

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