

3-Hydroxy fatty acids and branched GDGT-based proxy temperature calibrations for Southeast Asia

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ARTICLE INFO

Associate Editor: Melissa Berke

Keywords:

3-Hydroxy fatty acids
Branched GDGTs
Lake and soil sediments
Mean annual air temperature
pH
Southeast Asia

ABSTRACT

This study presents the first region-specific calibrations of 3-hydroxy fatty acids (3-OH-FAs), and branched glycerol dialkyl glycerol tetraethers (brGDGTs) for reconstructing temperature and hydroclimate across mainland Southeast Asia. Surface sediments and soils from forty-eight lakes and thirty catchments spanning tropical Thailand to temperate Yunnan, China, were analyzed to establish empirical relationships between biomarker indices and mean annual air temperature (MAAT). Among the 3-OH-FA indices, RAN₁₃ and the newly proposed RIN₁₅ exhibit the significant correlations with MAAT in lake sediments (slope = −0.026, $R^2 = 0.63$, $p < 0.001$ and slope = −0.162, $R^2 = 0.63$, $p < 0.001$, respectively), while RAN₁₅ performs best in soils (slope = −0.06, $R^2 = 0.83$, $p < 0.001$). For brGDGTs, the MBT_{5ME} index shows robust correlations with MAAT in lakes (slope = 0.026, $R^2 = 0.71$, $p < 0.001$), whereas the relationship is notably weaker in soils (slope = 0.013, $R^2 = 0.28$, $p < 0.01$), although it still outperforms MBT_{6ME}. pH-related indices (e.g., BR, RIN, CBT' and IR_{6ME}) display weaker relationships, reflecting limited pH variability across sites. Together, these results highlight distinct bacterial contributions to lipid assemblages and demonstrate that 3-OH-FA- and brGDGT-based temperature proxies respond systematically to regional thermal gradients. The new calibrations provide a robust framework for reconstructing, past temperature and hydroclimatic variability across Southeast Asia's monsoon region.

1. Introduction

The terrestrial environment plays a crucial role in shaping the impacts of climate change on human societies. In Asia, shifts in the monsoon system significantly influence temperature and precipitation patterns, directly affecting nearly half of the global population (Cook et al., 2010). The Southeast Asian monsoon, one of the largest and most complex monsoon systems on Earth, governs the circulation of heat and moisture during summer and brings cold, dry air in winter (Loo et al., 2015). This seasonal cycle shapes weather patterns, ecosystems,

and communities across tropical mainland Southeast Asia and extends to higher-latitude regions, including southwest China (Loo et al., 2015). Modern weather stations and satellites provide accurate climatic data, serving as essential tools for understanding decadal climate patterns and predicting future changes.

However, these instrumental records span only recent history (ca. 150 years) and are insufficient for capturing palaeoclimate variations over thousands of years to geological timescales. This leaves a critical data gap, as only palaeoclimate records cover the patterns of monsoonal system responses to forcing by anthropogenic warming. To address this

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<https://doi.org/10.1016/j.orggeochem.2026.105320>

Received 26 January 2026; Received in revised form 6 October 2026; Accepted 7 October 2026

Available online 13 October 2026

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limitation, organic geochemical proxies have been developed as key tools for reconstructing past climate conditions (Eglinton and Eglinton, 2008). Many of these proxies were originally developed for marine environments, and their application in lakes is often limited by proxy-specific constraints. For example, alkenone-based indices (e.g., U'_{37}) are not universally applicable due to the restricted occurrence of alkenone-producing organisms in many lake systems (Coolen et al., 2004; D'Andrea et al., 2006; Sun et al., 2007).

Over the past decade, glycerol dialkyl glycerol tetraethers (GDGTs) have been widely used as biomarkers for palaeoclimate reconstruction. Isoprenoid GDGTs, membrane lipids produced by certain Archaea, are characterized by an isoprenoid carbon skeleton ether-bonded to glycerol and containing 0–3 cyclopentane rings (Schouten et al., 2002). The TEX₈₆ palaeothermometer, based on the relative abundance of isoprenoid GDGTs, has proven effective in estimating lake temperatures only in very large lakes (e.g., the U.S. Great Lakes, Lake Malawi and Lake Tanganyika; Schouten et al., 2002, 2007; Powers et al., 2010; Tierney et al., 2010b). However, the reliability of TEX₈₆ decreases greatly in small to medium lakes such as those in this study (Powers et al., 2004; Tierney et al., 2010a). In contrast, branched GDGTs (brGDGTs), characterized by two alkyl chains ether-bonded to glycerol containing 4–6 methyl branches (Sinninghe Damsté et al., 2000), are better suited to typical lacustrine environments. Consequently, brGDGTs have become widely used biomarkers for terrestrial and lacustrine palaeoclimate reconstruction (e.g., Yang et al., 2015; Naafs et al., 2017a; Miller et al., 2018; Crampton-Flood et al., 2020; Raberg et al., 2022; Zhao et al., 2022; Wu et al., 2023). These bacterial lipids exhibit structural variations that reflect adaptations to environmental conditions (Wu et al., 2023). Their utility arises from homeoviscous adaptation, where bacteria modify methylation and cyclization to maintain membrane stability under changing temperature and pH (Halamka et al., 2023). A primary tool for identifying these sources is the Branched and Isoprenoid Tetraether (BIT) index, which compares the abundance of branched GDGTs to crenarchaeol (a lipid produced by aquatic Nitrososphaeria formerly known as Thaumarchaeota), helps distinguish between soil and aquatic organic matter sources (Hopmans et al., 2004). However, this indicator can be influenced by in situ production of GDGTs within lakes, complicating its interpretation (Zhu et al., 2025).

The Methylation (MBT) and Cyclization (CBT) indices were initially developed to reconstruct soil temperature and pH, respectively (Weijers et al., 2007), and have since been widely applied to estimate mean annual air temperature (MAAT) in lake sediments (Ceccopieri et al., 2019). However, improved chromatography revealed that 5- and 6-methyl isomers previously co-eluted, introducing uncertainty into early calibrations. This led to the development of refined, isomer-specific proxies. The MBT'_{5ME} index, based on 5-methyl brGDGTs, is now the standard lacustrine paleothermometer due to its strong temperature dependence and reduced pH influence (Wu et al., 2023). In contrast, the MBT'_{6ME} index, based on 6-methyl isomers shows stronger temperature correlations in specific settings, such as alkaline Chinese lakes and some marine sediments where 6-methyl-producing bacteria dominate (Pan et al., 2025), but it is generally more sensitive to non-thermal factors, including alkalinity, salinity, and aridity (Zhang et al., 2024). Environmental pH can be reconstructed using the cyclization-based CBT' index, which incorporates 6-methyl brGDGT isomers to improve accuracy in soil and lake calibrations (Ajallooeian et al., 2024). In addition, the isomer ratio IR_{6ME} provides an important diagnostic tool for assessing the reliability of temperature reconstructions derived from MBT'_{5ME} by quantifying the relative abundance of 6-methyl isomers (Wu et al., 2023). Elevated IR_{6ME} values (typically >0.4–0.5) indicate increased influence of non-thermal factors, such as salinity or oxygen limitation, which can lead to warm biases in reconstructed temperatures (Novak et al., 2025). It also functions as an indicator of alkalinity and salinity in aquatic systems (Bauersachs et al., 2024).

In recent years, 3-hydroxy fatty acids (3-OH-FAs) derived from the

outer membranes of Gram-negative bacteria have emerged as promising biomarker-based environmental proxies. These compounds, which range from C₁₀ to C₁₈, are primarily associated with lipid A in lipopolysaccharides (Wollenweber et al., 1982; Szponar et al., 2002). The potential use of 3-OH-FAs as palaeoclimate proxies has been explored in diverse environments, including soils (Wang et al., 2016, 2021; Yang et al., 2016; Huguet et al., 2019; Véquaud et al., 2021a, 2021b), speleothems (Blyth et al., 2006; Wang et al., 2018), aerosols (Lee et al., 2004), snow (Tyagi et al., 2015), lakes (Yang et al., 2021), and marine environments (Yang et al., 2020). Wang et al. (2016) initially developed temperature and pH proxies based on 3-OH-FA ratios in Chinese soils, introducing RAN₁₅ and RAN₁₇ as temperature proxies and several pH proxies including the ratio of the total sum of iso and anteiso 3-OH-FAs to the total amount of normal 3-OH-FAs (Branching Ratio), its negative logarithm (RIAN), the total sum of iso and anteiso 3-OH-FAs to the total amount of 3-OH-FAs (Branched Index), and the ratio between iso versus normal 3-OH-FAs (RIN). These soil-based indices were later applied to stalagmites, producing temperature and hydrological reconstructions (Wang et al., 2018), and verified across additional soil transects in Tanzania and Italy, confirming the promise of these proxies (Huguet et al., 2019). Global calibrations for temperature and pH using 3-OH-FAs were further refined using linear equations and machine learning models (Huguet et al., 2019; Véquaud et al., 2021b; Wang et al., 2021). The RAN₁₃ index, derived from 3-OH-FAs, has been proposed as a novel proxy for sea surface temperatures (Yang et al., 2020) and shows a moderate correlation with MAAT in lake sediments (Yang et al., 2021). Despite their promising applications, the use of GDGT- and 3-OH-FA-based proxies for reconstructing climate from lake archives faces several challenges. These include uncertainties about the sources of these compounds, the influence of local environmental factors such as pH and temperature on their distribution, and the need for more region-specific calibrations to improve the accuracy of palaeoclimate reconstructions (Schouten et al., 2013). Continued research and global calibration efforts are essential to enhance the reliability and applicability of these proxies in diverse environments.

This study investigates the potential of GDGT- and 3-OH-FA-based proxies in lake environments, with a focus on lakes ($n = 48$) and surrounding soils ($n = 30$) across Southeast Asia. The study area spans a latitudinal transect from southwestern China to the southern peninsula of Thailand, characterized by high variability in precipitation (600–3000 mm p.a.) due to the influence of the Asian monsoon. This region experiences a transition from tropical climates to semi-arid and sub-tropical climates (MAAT: 11 °C–28 °C), offering a unique opportunity to explore how environmental shifts impact bacterial biomarkers. By analyzing the distribution of both GDGTs and 3-OH-FAs, along with associated bacterial gene compositions, we propose novel temperature proxies for both lake and soil systems in the Southeast Asian region. The study aims to evaluate the performance of these proxies against established GDGT-based proxies and instrumental records, while also assessing whether these biomarkers reflect bacterial physiological responses to environmental changes, providing new insights into the use of biomarkers in regions with diverse climatic conditions.

2. Methods

2.1. Sampling region and sites

The Southeast Asia region features diverse environmental parameters due to varying altitudes, latitudes, and climatic conditions. Here, we define Southeast Asia, geographically, to include representative samples from Indochina (Thailand and Vietnam), but we also extend into the neighbouring Yunnan Province of southern China. This was undertaken to ensure a broad climatic gradient for proxy calibration. The inclusion of samples from Yunnan ensured a range of much cooler lake and soil mean annual temperatures that are not represented in Thailand and Vietnam. Our study region spans low-lying coastal areas to mountainous

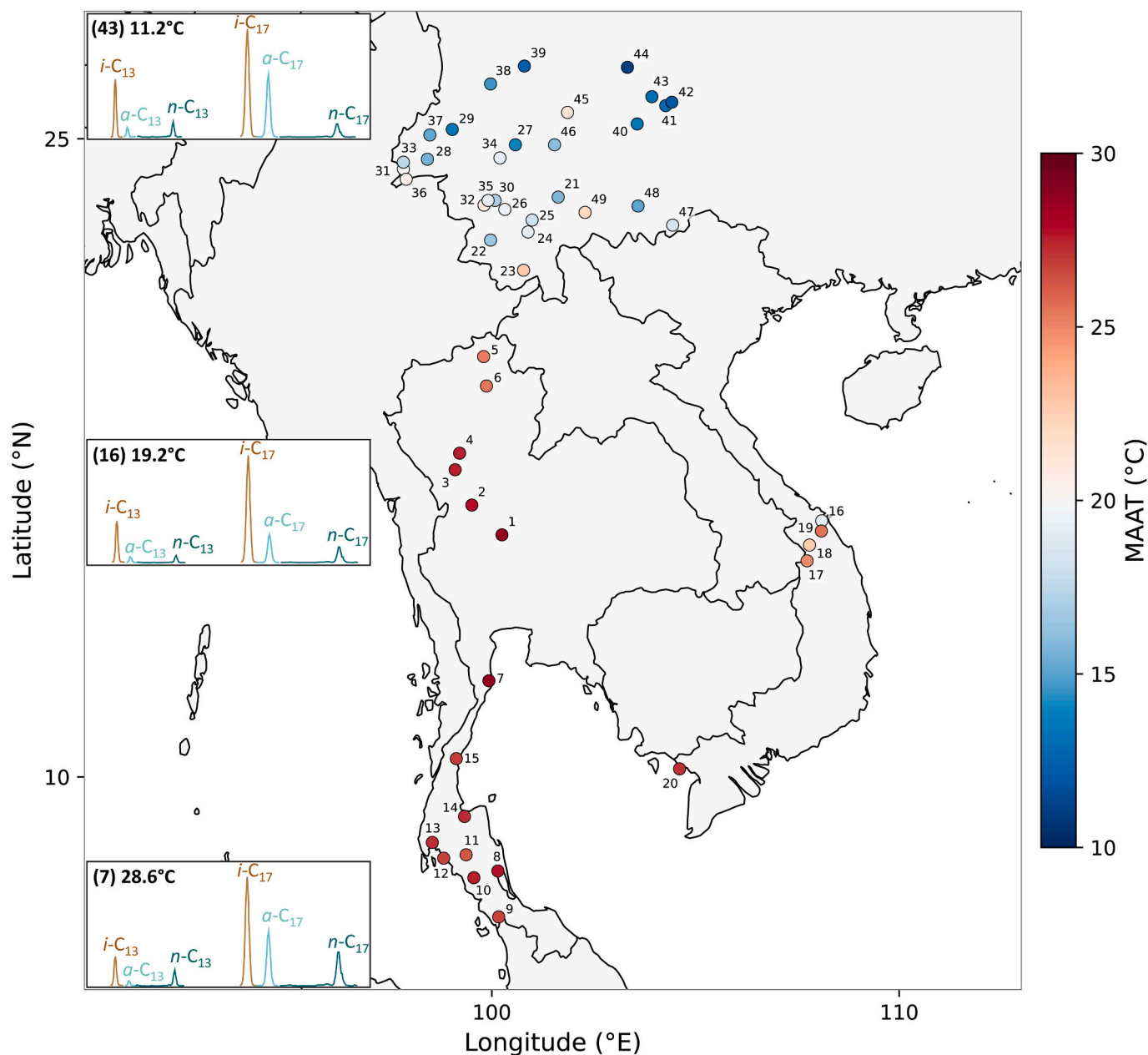


Fig. 1. Locations of the 48 Southeast Asian lakes used in this study (Supplementary Data). Paired soil catchment and lake surface sediment samples were collected from the Thai and Vietnamese lakes, except southwest China contains only lake sediments. The colour of the dots shows the mean annual air temperature (MAAT) of each sampling site. The three inset boxes give examples of the partial distributions of the C_{13} range of study sites, and C_{17} 3-OH-FA homologs from lake sites with the lowest, medium, and highest MAATs (11.2–28.6 °C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

terrains, with altitudes ranging from sea level to over 2500 m above sea level in parts of Yunnan Province, Southwest China (Fig. 1). Meteorological and satellite data over the past 30 years for the region show annual average temperatures and annual average precipitation are highly variable, with the former spanning 11–28 °C and the latter 600–3000 mm. Climatic conditions across the region are influenced by latitude and geography, with distinct patterns observed from southern Thailand to northern mainland Southeast Asia. The southern peninsular region of Thailand experiences a wetter climate due to the dominance of the summer monsoon, while central Vietnam, shielded by mountain ranges, remains comparatively drier. Yunnan Province in Southwest China, situated at higher altitudes, experiences cooler temperatures and seasonal variations driven by the interaction between monsoonal systems and continental air masses.

2.2. Sample collection

In December 2021, sediments and surrounding soils were systematically collected along a latitudinal transect in Thailand, spanning fifteen lakes from Nong Bueng in the north to Thale Bun in the south (Fig. 1). The sampling sites covered an annual temperature range of 25 °C–28 °C, with annual precipitation varying from 900 mm to over 2000 mm (Supplementary Data). Additional sampling campaigns were conducted in March 2023 across central and southern Vietnam, with paired soil and lake sediment samples from four central Vietnamese lakes across and altitudinal transect in Ngoc Linh Mountains, capturing an environmental gradient with annual temperatures ranging across 15 °C–26 °C and annual precipitation from 1900 mm to 2500 mm. Collaborative research in Yunnan Province, China, provided 29 lake (reservoir) sediment

samples from sites with annual temperatures between 11 °C and 21 °C and annual precipitation between 600 mm and 1600 mm. In the field, lake surface sediments were collected using an Ekman grab, and surrounding soil samples adjacent to lake catchments were obtained by removing the top 0–5 cm of soil, along with any surface debris. Additionally, subsamples of soil or sediment were transferred to lysis tubes containing DNA/RNA Shield (Zymo Research, California) and stored at –20 °C to preserve DNA for bacterial community analysis. Environmental parameters, including lake depth, sediment temperature, pH, dissolved oxygen, water temperature, and water pressure were recorded using multiprobe instruments and portable meters. Additionally, soil temperature was recorded during collection using a Traceable® Lollipop Water-Resistant thermometer. Soil moisture was also determined by comparing fresh and freeze-dried weights.

2.3. pH measurement

PH was measured following the method of Wang et al. (2016) and Yang et al. (2021). After freeze-drying, the samples were mixed with Milli-Q deionized (DI) water at a ratio of 1:2.5 (g/ml). The mixture was left to stand for 20 min, after which the supernatant was measured using a pH meter with a precision of ± 0.01 . Each sample was measured 3 times, and the final pH value was calculated as the mean of these measurements.

2.4. 3-OH-FA and GDGT extractions

The samples were stored at –20 °C in the laboratory before being pre-weighed and freeze-dried to remove moisture. Once dried, they were ground into a fine powder using a pestle and mortar. Lipid extraction followed the method of Wang et al. (2016) and Yang et al. (2021), with modifications to the acid-methylation process. First, 10 g of each sample were digested in 30 ml of a 12 M HCl and methanol (MeOH) mixture in a 1:3 ratio. The solutions were vortexed for thorough mixing and then heated at 70 °C for 14 h. After cooling, dichloromethane (DCM) was added for extraction, followed by vortexing and sonication for 20 min. The solutions were then centrifuged at 3000 rpm for 3 min to separate the supernatant, which contained the methylated total lipid extract (TLE). This extraction process was repeated 4 times to maximize TLE yield. The methylated TLEs were concentrated using a rotary evaporator and dried under a gentle nitrogen (N_2) flow. In this study, we found that the methylated TLEs could be directly analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) after derivatization with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). The samples were heated at 70 °C for 1.5 h before GC-MS analysis, which was performed within 24 h to ensure optimal results.

For brGDGT extraction, 10 g of freeze-dried sediment was placed into 30 mL test tubes with dichloromethane (DCM, High-Performance Liquid Chromatography (HPLC) grade), vortexed, heated to 40 °C for 1 h, sonicated for 20 min, and repeated the extraction for four times to yield TLE. The extracts were evaporated under N_2 to obtain dried TLEs, which were subsequently passed through a Na_2SO_4 column to remove residual water and particulates. Lipid fractions were then separated by silica gel chromatography (Silica 60A, 30–75 μm , Davisil®) and sequentially eluted with: (1) *n*-hexane to obtain *n*-alkanes, (2) *n*-hexane:DCM (2:1 v/v) to obtain alcohols, (3) DCM to obtain aldehydes, and (4) DCM: methanol (95:5(MeOH) v/v) to obtain polar compounds. brGDGTs were recovered from the polar fraction, which was re-dissolved in *n*-hexane: isopropanol (99,1 v/v) and filtered (0.45 μm PTFE) prior to analysis.

2.5. DNA extraction, 16S rRNA gene sequencing and bacterial community analysis

Genomic DNA was extracted from 250 mg of homogenized soil or sediment using the DNeasy PowerSoil Pro kit (Qiagen, Germany) following the manufacturer's protocol. Purified DNA extracts were

stored at –20 °C until further processing. The V4 region of the bacterial 16S rRNA gene was amplified by PCR using the 515F and 806R primer pair (forward: 5'-GTGCCAGCMGCCGCGGTAA-3'; reverse: 5'-GGACTACHVGGGTWTCTAAT-3') as described by Caporaso et al. (2011). PCR amplification and library preparation were carried out by the Envision Team at the University of Birmingham. Paired-end sequencing (2 × 300 bp) was performed by BGI Genomics Co., Ltd. using the DNBSEQ platform. The 16S rRNA gene sequences were processed according to the DADA2 pipeline (Callahan et al., 2016). Following sample demultiplexing, primers were removed, and reads were trimmed to the position where the quality score dropped below Q30 (forward: 300 bp; reverse: 220 bp). High-stringency filtering was performed, allowing a maximum of 2 expected errors and no ambiguous bases. Filtered forward and reverse reads were dereplicated into unique sequence variants and merged with a minimum overlap of 12 bases. An amplicon sequence variant (ASV) table was generated, and taxonomy was assigned to each ASV using the SILVA database v.138.1 (Quast et al., 2013) with a minimum bootstrap confidence of 60. Non-bacterial and unclassified sequences were removed. Samples were normalized by rarefaction to a uniform sequencing depth of 10,000 reads, selected based on the depth at which richness plateaued for most samples (two samples below this threshold were excluded). This resulted in a total of 75,755 bacterial ASVs across 31 samples (14 lake sediments and 17 soils) for downstream analysis. The abundance of each ASV was converted to relative abundance per sample. Bacterial community composition was analyzed using the Phyloseq R package and visualized for the top 15 most abundant bacterial phyla (McMurdie and Holmes, 2013).

2.6. Instrumentation

The methylated TLEs from the acid-methylation fractions were analyzed using Agilent 7890B gas chromatograph coupled with a 5977A mass spectrometer, equipped with a BP5ms fused silica capillary column (60 m × 0.32 mm internal diameter × 0.25 μm film thickness) at the University of Birmingham. The GC oven temperature was initially set at 60 °C and held for 1 min before increasing to 200 °C at a rate of 10 °C/min, then to 300 °C at 2 °C/min, where it was held for 15 min. The total run time was 80 min. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. The mass spectrometer operated with an ionization energy of 70 eV. The 3-OH-FAs were identified based on their mass spectra and relative retention times. The trimethylsilyl (TMSi) esters of 3-OH-FAs exhibited characteristic fragment ions, including m/z 175 ($[CH_3]_3SiO=CHCH_2CO_2CH_3$)⁺, resulting from cleavage between C_3 and C_4 , and a base peak of M^+-15 , corresponding to the loss of a CH_3 group. Additional diagnostic ions included m/z 103, 89, 133, 159, and M^+-31 (Wang et al., 2016). Each sample was analyzed in duplicate or triplicate to ensure accuracy and standard deviation of the indices were assessed accordingly.

BrGDGTs were analyzed at the Key Laboratory of Western China's Environmental Systems (MOE), Lanzhou University. The analysis was performed using an Agilent 1290 II series ultra-performance liquid chromatography coupled with an atmospheric pressure chemical ionization 6465B triple quadrupole mass spectrometer (UPLC-APCI-MS/MS). A flow rate of 0.3 mL/min was applied, and an aliquot of 15 μl was injected and separated using two Hypersil Gold Silica columns in sequence (each 150 mm × 2.1 mm × 1.9 μm ; Thermo Fisher Scientific, Lithuania), maintained at 40 °C. GDGTs were eluted isocratically with 84% solvent A and 16% solvent B for the first 5 min (where A = *n*-hexane, B = ethyl acetate), followed by a linear gradient to 82% A and 18% B from 5 to 65 min, then linearly changed to 100% B over 10 min, maintained at 100% B for 5 min to wash the system, and finally returned to 84% A and 16% B to equilibrate the pressure. Analyses were conducted in selective ion monitoring (SIM) mode to track m/z 1302, 1300, 1298, 1296, 1292, 1050, 1048, 1046, 1036, 1034, 1032, 1022, 1020, 1018, and 744 (C_{46} standard) for the full suite of commonly analyzed GDGTs.

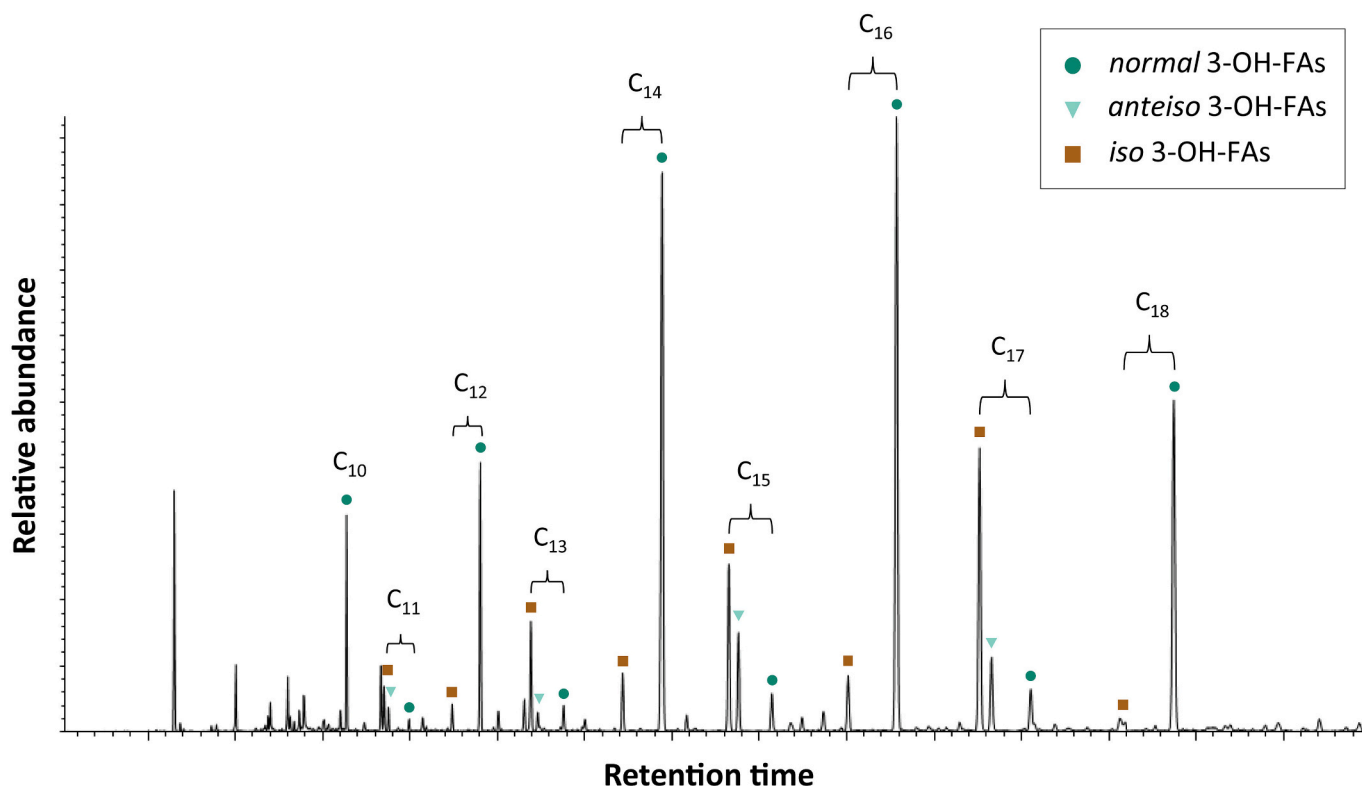


Fig. 2. An example GC-MS trace (175 m/z) showing a typical distribution of 3-OH-FAs in the lake surface sediment sample (Truoc Dong Reservoir).

2.7. Equations of 3-OH-FA and brGDGT indices

3-OH-FAs can be grouped by their molecular structure into straight-chain (*normal* (n -)) and branched isomers (*iso* (i -), and *anteiso* (a -)), depending on the presence and position of a terminal methyl branch. These compounds are predominantly saturated, and differences in their relative abundances form the basis of widely used indices for environmental interpretation. 3-OH-FA-based temperature indices, including the ratio of *anteiso* to *normal* C_{13} (RAN_{13} ; Eq. (1)) and the ratio of *iso* to *normal* C_{17} (RIN_{17} ; Eq. (2)) were proposed as temperature calibrations for lake sediments (Yang et al., 2021). In addition, the ratio of *anteiso* to *normal* C_{15} (RAN_{15} ; Eq. (3)) and the ratio of *anteiso* to *normal* C_{17} (RAN_{17} ; Eq. (4)) were proposed as temperature calibrations for soils (Wang et al., 2016). In this study, we also propose the ratio of *anteiso* to *normal* C_{15} (RIN_{15} ; Eq. (5)) as a novel index for lake sediment in Southeast Asia.

$$RAN_{13} = a - C_{13}/n - C_{13} \quad (1)$$

$$RIN_{17} = i - C_{17}/n - C_{17} \quad (2)$$

$$RAN_{15} = a - C_{15}/n - C_{15} \quad (3)$$

$$RAN_{17} = a - C_{17}/n - C_{17} \quad (4)$$

$$RIN_{15} = i - C_{15}/n - C_{15} \quad (5)$$

In addition, 3-OH-FA-based pH indices were calculated following Wang et al. (2016), which were related to soil pH. Branching Ratio is the ratio of the total sum of *iso* and *anteiso* 3-OH-FAs to the total amount of *normal* 3-OH-FAs (Eq. (6)). RIAN is a negative logarithm of the Branching Ratio (Eq. (7)). The Branched Index (BI) is the ratio of the total sum of *iso* and *anteiso* 3-OH-FAs to the total amount of 3-OH-FAs (Eq. (8)). RIN is the ratio between *iso* versus *normal* 3-OH-FAs (Eq. (9)). In addition, these indices were used to calibrate with lake water pH (Yang et al., 2021).

$$\text{Branching Ratio} = (I + A)/N \quad (6)$$

$$\text{RIAN} = -\log [(I + A)/N] \quad (7)$$

$$\text{Branched Index} = (I + A)/(I + A + N) \quad (8)$$

$$\text{RIN} = I/N \quad (9)$$

where I represents the sum of all the *iso* 3-OH-FAs, A represents the sum of all the *anteiso* 3-OH-FAs, and N represents the sum of all *normal* 3-OH-FAs.

In addition to brGDGT indices, the Branched and Isoprenoid Tetraether (BIT) index was introduced by Hopmans et al. (2004) to differentiate between soil-derived and aquatic organic matter sources by comparing the abundance of brGDGTs to crenarchaeol, a lipid produced by aquatic Nitrososphaeria (formerly Thaumarchaeota; Eq. (10)). The MBT'_{5ME} index (Eq. (11)), using exclusively 5-methyl brGDGTs, is the standard lacustrine paleothermometer with a robust, near-universal relationship with temperature (Raberg et al., 2022). In contrast, the MBT'_{6ME} index, which incorporates 6-methyl brGDGTs, is often more sensitive to non-thermal parameters such as alkalinity, salinity, and aridity (Dang et al., 2018; Eq. (12)), though we calculate it here for comparison to MBT'_{5ME} .

$$\text{BIT} = \frac{Ia + IIa + IIIa + IIa' + IIIa'}{Ia + IIa + IIIa + IIa' + IIIa' + \text{Crenarchaeol}} \quad (10)$$

$$\text{MBT}'_{5ME} = \frac{Ia + Ib + Ic}{Ia + Ib + Ic + IIa + IIb + IIc + IIIa} \quad (11)$$

$$\text{MBT}'_{6ME} = \frac{Ia + Ib + Ic}{Ia + Ib + Ic + IIa' + IIb' + IIc' + IIIa'} \quad (12)$$

To reconstruct environmental pH, we calculate the CBT' index, a refined cyclization metric proposed by De Jonge et al. (2014a) that incorporates 6-methyl isomers to improve reconstruction accuracy in

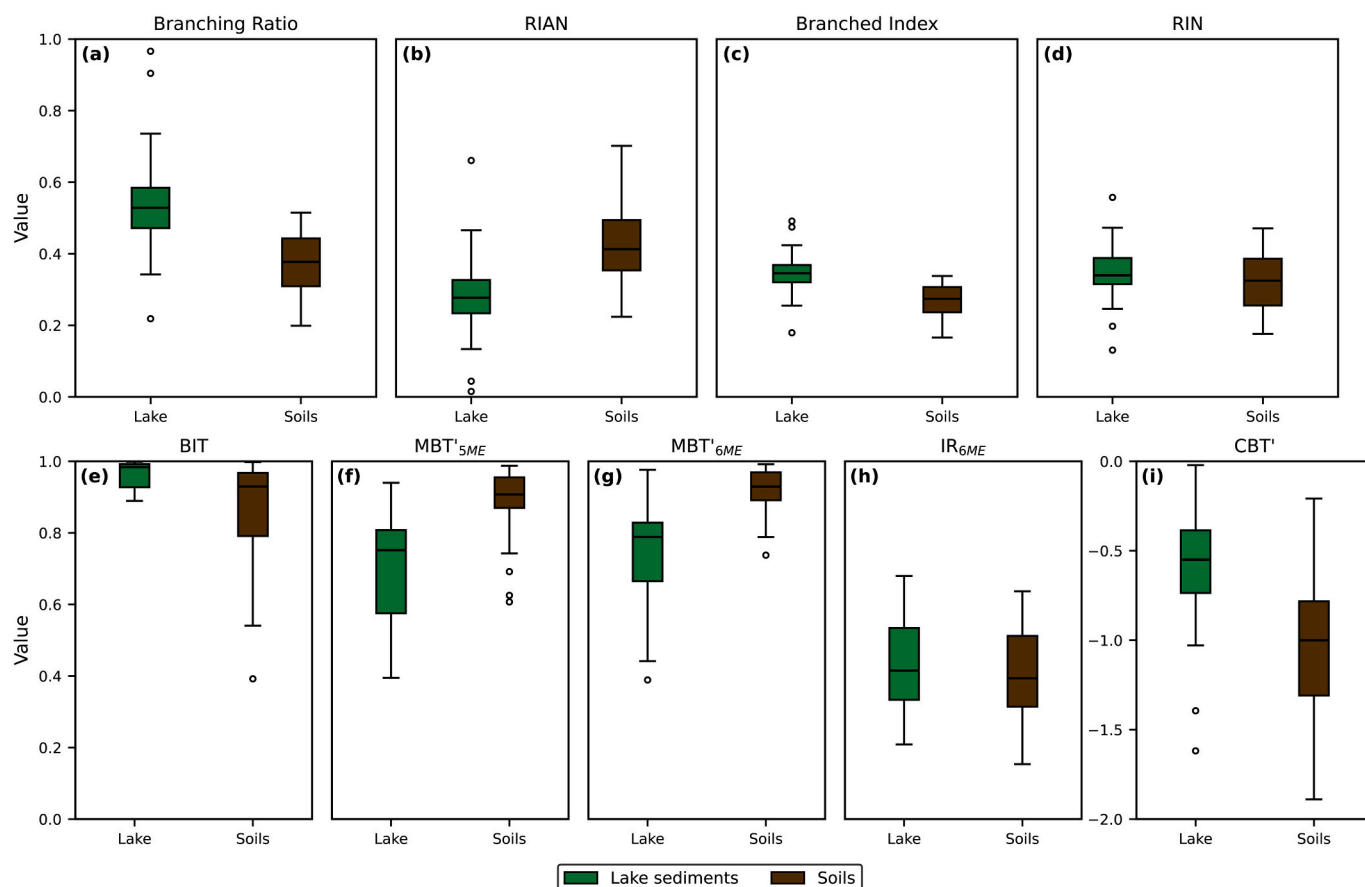


Fig. 3. Box-and-whisker plots of 3-OH-FA-based indices, (a) the Branching Ratio (BR), (b) RIAN, (c) the Branched Index (BI), (d) RIN, and brGDGT-based indices (e) BIT, (f) MBT'_{5ME}, (g) MBT'_{6ME}, (h) IR_{6ME}, and (i) CBT'.

global soil and lake calibrations (Eq. (13)). In addition, the Isomer Ratio of 6-methylated brGDGTs (IR_{6ME}) serves as a critical diagnostic tool to assess the validity of temperature reconstructions derived from MBT'_{5ME}. It quantifies the relative proportion of 6-methyl isomers within the total pool of pentamethylated and hexamethylated structures (Eq. (14)).

$$CBT' = \log_{10} \left(\frac{Ic + IIa' + IIb' + IIc' + IIIa' + IIIb' + IIIc'}{Ia + IIa + IIIa} \right) \quad (13)$$

$$IR_{6ME} = \frac{IIa' + IIb' + IIc' + IIIa' + IIIb' + IIIc'}{IIa + IIb + IIc + IIa' + IIb' + IIc' + IIIa + IIIb + IIIc + IIIa' + IIIb' + IIIc'} \quad (14)$$

In this study, the analysis focused on the relative abundances of 3-OH-FAs and brGDGTs; absolute concentrations were not determined.

3. Results

3.1. Environmental parameters

This study analyzes co-sampled lake surface sediments and surrounding soils collected across Southeast Asia and southwest China, including 19 lakes and 19 soils from southern Thailand to central Vietnam, 11 additional soils from an elevational transect on Ngoc Linh Mountain, and 29 reservoir sediments from Yunnan, China, comprising a total dataset of 48 lakes and 30 soils. Study sites span altitudes from 0 to 2548 m above mean sea level, water depths from 1.7 to 33.3 m, and lake surface areas from 2300 to 212,380,000 m². Field and laboratory measurements showed lake water pH values range from 6.3 to 9.3, soil pH from 4.6–8.2, and dissolved oxygen concentrations from 1.44 to

7.41 mg/L. Satellite-derived climate data (WorldClim) indicate MAAT range from 11.1 to 28.6 °C and mean annual precipitation (MAP) from 667 to 2950 mm (Fick and Hijmans, 2017; Fig. 1 and Supplementary Data).

3.2. 3-OH-FA distributions and indices

3-OH-FA distributions in both lake sediments and soils span C₁₀–C₁₈, with C₁₆ generally being the dominant homolog, although some sites are characterized by C₁₄ dominance. Odd-numbered 3-OH-FAs are enriched in *iso* and *anteiso* homologs, whereas even-numbered compounds are predominantly *normal* 3-OH-FAs (Fig. 2). However, even-numbered unsaturated 3-OH-FAs are deficient compared to alkaline lakes in Central China (Yang et al., 2021). 3-OH-FA-based indices also differ between lake sediments and soils, as shown in the box-and-whisker plots (Fig. 3). Lake sediments exhibit higher and wider ranges of the Branching Ratio (BR; 0.22–0.97; mean = 0.54) compared to soils (0.20–0.51; mean = 0.37; Fig. 3a), with the difference being statistically significant ($p < 0.001$). RIAN is a negative logarithm of the Branching Ratio, was significantly lower in lake sediments (0.02–0.66; mean = 0.28) compared to soils (0.22–0.70; mean = 0.44; $p < 0.001$; Fig. 3b). Similarly, the Branched Index (BI) values was significantly higher in lake sediments (0.18–0.49; mean = 0.35) than in soils (0.17–0.34; mean = 0.27; $p < 0.001$; Fig. 3c). In contrast, RIN showed similar values between lake sediments (0.13–0.56; mean = 0.35) and soils (0.18–0.47; mean = 0.32; Fig. 3d), with no statistically significant difference ($p = 0.34$). Furthermore, temperature-related 3-OH-FA indices also show distinct distributions between lake sediments and soils. In lake sediments, RAN₁₃ ranges from 0.15 to 0.95 with a mean value of 0.52, while RIN₁₇ ranges from 1.47 to 9.02 with a mean of 5.61.

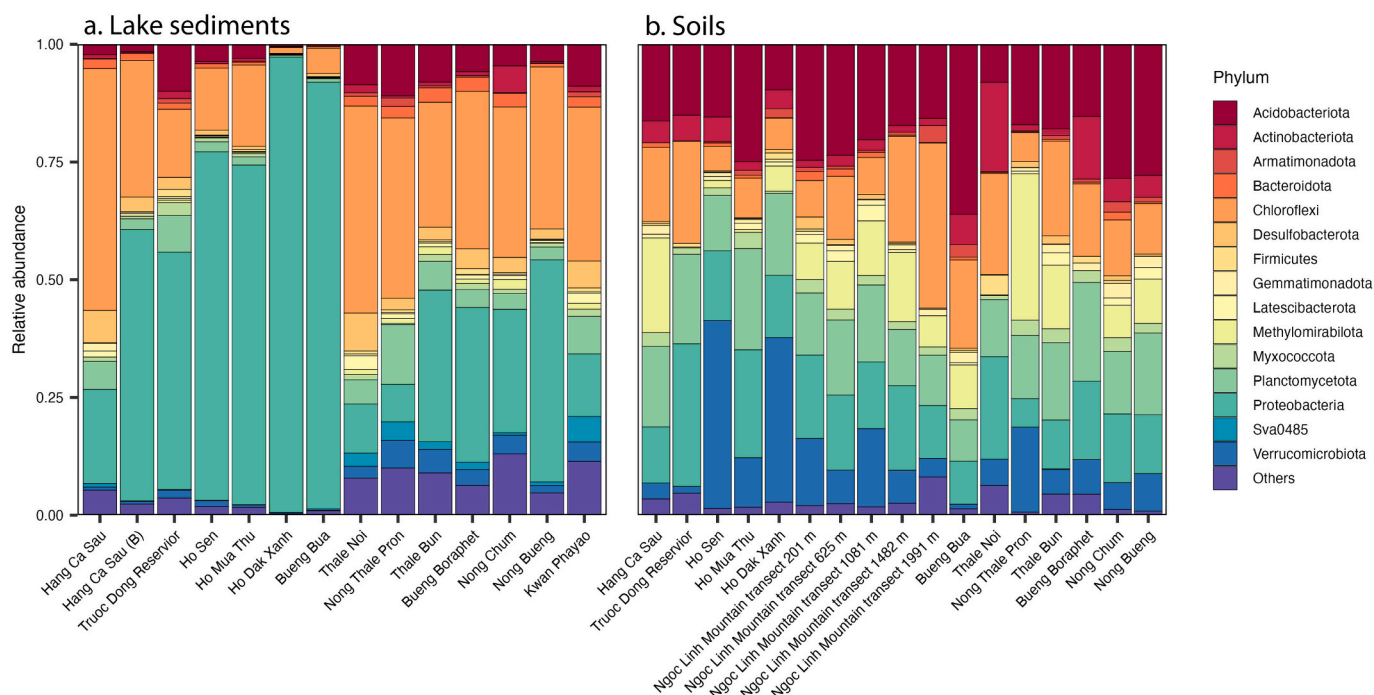


Fig. 4. Phylum composition of Gram-negative bacterial community from (a) lake surface sediment samples ($n = 14$), and (b) soil samples ($n = 18$).

In soils, RAN_{15} ranges from 0.59 to 3.05 with a mean value of 1.75, whereas RAN_{17} ranges from 0.41 to 2.56 with a mean of 1.08 (Supplementary Data).

3.3. BrGDGT distributions and indices

BrGDGT distributions in both lake sediments and soils contain the common suite of tetramethylated (series I), pentamethylated (series II), and hexamethylated (series III) brGDGT isomers. Tetramethylated brGDGTs dominate in both environments, although they are relatively more abundant in soils (49.92–97.76%; mean = 82.12%) than in lake sediments (24.57–91.90%; mean = 57.14%). In contrast, pentamethylated compounds are enriched in lake sediments (7.83–55.91%; mean = 34.95%) relative to soils (2.24–42.75%; mean = 16.57%). Hexamethylated compounds are generally minor constituents but also show higher abundances in lake sediments (0.27–26.14%; mean = 7.90%) compared to soils (0–7.33%; mean = 1.31%). The 5-methyl brGDGTs consistently dominate over 6-methyl isomers in both lake sediments and soils. In lake sediments, 5-methyl compounds average 34.95%, whereas 6-methyl compounds average 7.90%. Similarly, soils show lower abundances of 6-methyl isomers, with mean values of 16.57% for 5-methyl and 1.31% for 6-methyl compounds (Supplementary Data).

BrGDGT-derived indices also exhibit clear differences between lake sediments and soils. The BIT index ranges from 0.89 to 1.00 (mean = 0.96) in lake sediments and from 0.25 to 1.00 (mean = 0.83) in soils, with a significant difference between the two environments ($p < 0.05$), indicating the organic matter is derived from terrestrial sources (Fig. 3e). The brGDGT-based temperature index also differed significantly between lake sediments and soils. MBT'_{5ME} index ranges from 0.39 to 0.94 (mean = 0.70) in lake sediments and from 0.63 to 0.96 (mean = 0.83) in soils ($p < 0.001$; Fig. 3f), while MBT'_{6ME} values range from 0.39 to 0.98 (mean = 0.74) in lake sediments and from 0.79 to 0.91 (mean = 0.86) in soils ($p < 0.001$; Fig. 3g). The IR6ME values, which have been associated with environmental pH, are similar between lake sediments (0.21–0.68; mean = 0.43) and soils (0.25–0.64; mean = 0.43; Fig. 3h) with no significant difference ($p = 0.569$). In contrast, CBT' values are generally higher in lake sediments (−1.62 to −0.02;

mean = −0.56) than in soils (−1.89 to −0.21; mean = −1.09; Fig. 3i), with a significant difference between the two environments ($p < 0.001$).

3.4. Bacterial community composition

Bacterial community composition in lake sediments (Fig. 4a) was dominated by Proteobacteria and Chloroflexi, though the relative contribution of each phylum varied among lakes. Proteobacteria were consistently abundant across nearly all lake sediments, most notably in Ho Sen, Ho Mau Thu, Ho Dak Xanh (Central Vietnam), and Bueng Bua (South Thailand), where Proteobacteria comprised >70% of the community. Chloroflexi reached particularly high relative abundances (up to 51%) in several lakes and dominated the community in Hang Ca Sau (South Vietnam), Thale Noi, Nong Thale Pron (South Thailand), Nong Chum, and Kwan Phayao (North Thailand). Other members of the lake sediment community included Planctomycetota (up to 13%), Acidobacteriota (up to 11%), Desulfobacterota (up to 8%), and Verrucomicrobiota (up to 6%).

Soils exhibited a markedly different community structure compared to lake sediments (Fig. 4b), with Verrucomicrobiota and Acidobacteriota emerging as a dominant or co-dominant phyla across most sites. Verrucomicrobiota dominated Ho Sen and Ho Dak Xanh soils in Central Vietnam (40% and 35%, respectively), while Acidobacteriota dominated Bueng Bua soils in South Thailand (36%). Proteobacteria and Chloroflexi remained abundant in soils but were generally less dominant than in lake sediments (<35% and <30%, respectively). Other bacterial phyla also comprised a considerable proportion of catchment soil communities, such as Methylomirabilota (up to 31%), Planctomycetota (up to 22%), and Actinobacteriota (up to 19%).

4. Discussion

4.1. Comparison of 3-OH-FA and brGDGT distributions in lake sediments and soils

To characterize the distributions of 3-OH-FAs in various environmental settings, we follow the method established by (Yang et al., 2020) and employ a ternary diagram, which plots the sum of anteiso 3-OH-FAs,

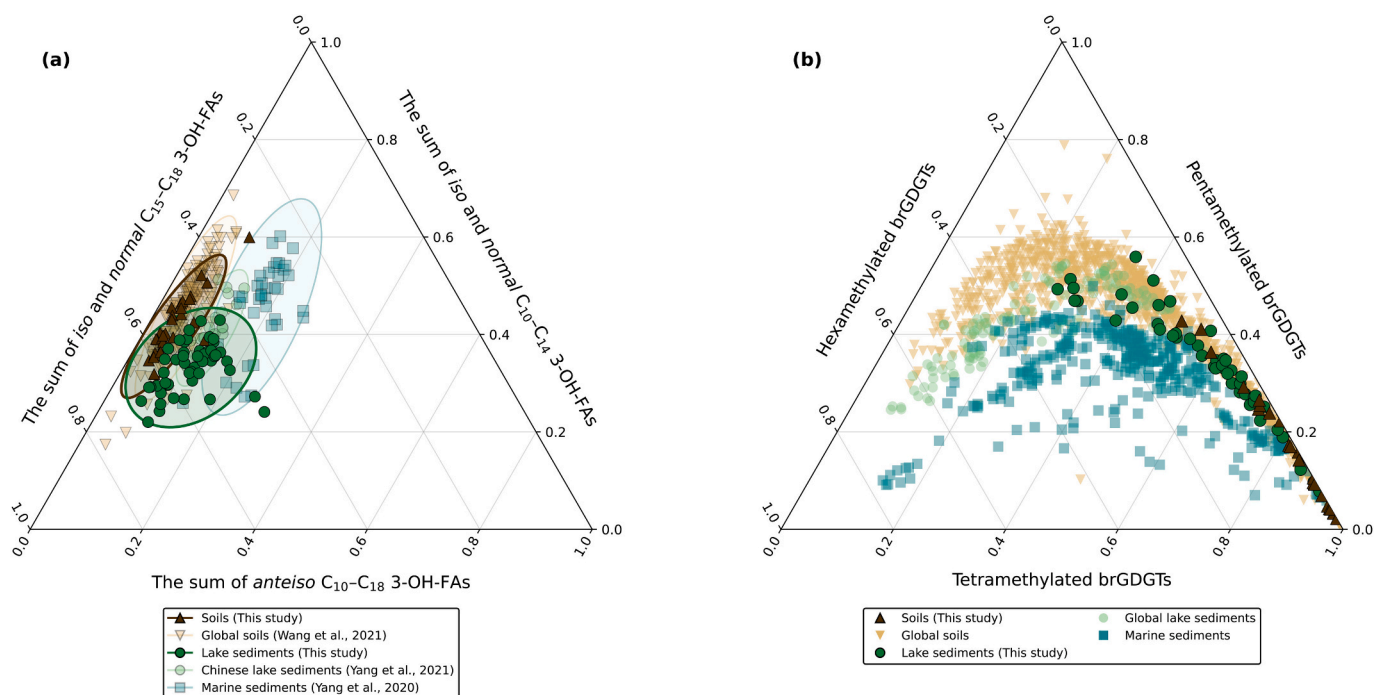


Fig. 5. Ternary plot showing (a) relative abundances of 3-OH-FA homologs in global soils (Wang et al., 2021), Chinese lake sediments (Yang et al., 2021), marine sediments (Yang et al., 2020), and lake surface sediments and soil samples in this study. The ternary division is based on the sum of *anteiso* 3-OH-FAs, the sum of *iso* and *normal* C₁₀-C₁₄ 3-OH-FAs, and the sum of *iso* and *normal* C₁₅-C₁₈ 3-OH-FAs, and (b) relative abundances of tetramethylated, pentamethylated, and hexamethylated brGDGTs in global soils (Xiao et al., 2020; Raberg et al., 2022), lake sediments (Dang et al., 2018; Raberg et al., 2021; Lei et al., 2023; Bauersachs et al., 2024; Hou et al., 2026), marine sediments (Warden et al., 2016; van Soelen et al., 2017; Raberg et al., 2022) and lake surface sediments and soil samples in this study.

the sum of *iso* and *normal* homologs from C₁₀ to C₁₄, and the sum of *iso* and *normal* homologs from C₁₅ to C₁₈. Fig. 5a illustrates the relative abundance of 3-OH-FAs in this study compared with published data, including global soils, lake sediments, and marine sediments (Wang et al., 2016, 2021; Huguet et al., 2019; Yang et al., 2020, 2021). The ternary diagram shows that lake sediment samples plot between soils and marine sediments (Fig. 5a). Lake sediments have a relatively higher *anteiso* content than soils but lower than marine sediments. The sum of *iso* and *normal* 3-OH-FAs from C₁₀ to C₁₄ is also lower in lake sediments than in marine sediments, but aligns more closely with soils. However, the abundance of the sum of *iso* and *normal* 3-OH-FAs from C₁₅ to C₁₈ in lake sediments is similar to that in soils but relatively higher than in marine sediments (Fig. 5a). Furthermore, lake sediments contain a higher *anteiso/normal* ratio whilst soils exhibit this ratio in lower amounts, which may reflect differences between terrestrial and aquatic sources of bacterial 3-OH FAs (Yang et al., 2021). However, the relative abundance of unsaturated 3-OH-FAs in our study sites differs from previous findings, which reported low unsaturated abundance in both lake and marine sediments (Yang et al., 2020, 2021). This discrepancy may be attributed to site-specific factors influencing independent compound production. Overall, the distribution of 3-OH-FAs in lake sediments reflects a complex interplay between terrestrial inputs, primarily derived from soils (characterized by a low *anteiso* proportion), and in situ aquatic production, which is more dominant in marine sediments (characterized by a high *anteiso* proportion). Superimposed on these 3-OH-FA distributional clusters, which partly indicate provenance, is the effect of measurable environmental factors (e.g., temperature and pH), which stimulate a physiochemical response to maintain membrane fluidity and permeability (Kaneda, 1991; Yoon et al., 2015).

The bacterial communities in lake sediments were predominantly composed of Proteobacteria and Chloroflexi, consistent with previous observations in Chinese lakes (Wang et al., 2025), and globally (Li et al., 2024). Planctomycetota, Acidobacteriota, Desulfobacterota, and Verrucomicrobiota were also present across lake sediments but rarely

dominated the community. In contrast, catchment soils showed a more diverse bacterial profile, with Acidobacteriota and Verrucomicrobia as co-dominant phyla alongside substantial contributions from Proteobacteria, Chloroflexi, Methylomirabilota, Planctomycetota, and Actinobacteriota, reflecting typical soil bacterial assemblages (Dedysh and Sinninghe Damsté, 2018; Yang et al., 2020).

The compositional differences between lake sediments and soils likely contribute to the observed variability in 3-OH-FA distributions between the two environments, and highlight the influence of depositional setting on biomarker source inputs. For example, the higher *anteiso/normal* ratio in lake sediments may reflect a greater contribution from aquatic bacteria, particularly members of Desulfobacterota and Chloroflexi, which were consistently more abundant in lake sediments than in soils (Yang et al., 2021, 2025). Conversely, the higher relative abundance of *normal* 3-OH FAs in soils may be attributed to production by bacterial taxa characteristic of soils, such as members of Acidobacteriota and Verrucomicrobiota. However, the bacterial sources of 3-OH FAs remain poorly constrained, with experimental evidence limited to a small number of cultured strains (Hellequin et al., 2023), and the systematic relationship between 3-OH FAs and bacterial community composition requires further investigation through paired 3-OH FA and amplicon sequencing analyses spanning a broader range of habitat types and capturing a wider diversity of bacteria.

The brGDGT ternary diagram (Fig. 5b) illustrates the relative proportions of tetramethylated, pentamethylated, and hexamethylated brGDGTs in lake sediments and soils from this study, compared with previously reported global soils (e.g., Xiao et al., 2020; Raberg et al., 2022), marine sediments (e.g., Warden et al., 2016; van Soelen et al., 2017; Raberg et al., 2022), and lake sediments (e.g., Dang et al., 2018; Raberg et al., 2021; Lei et al., 2023; Bauersachs et al., 2024; Hou et al., 2026). The brGDGT distributions of lake sediments from this study plot between the global soil and marine sediment endmembers, suggesting mixed terrestrial and aquatic contributions to sedimentary brGDGTs. These lake sediment samples also overlap with previously reported

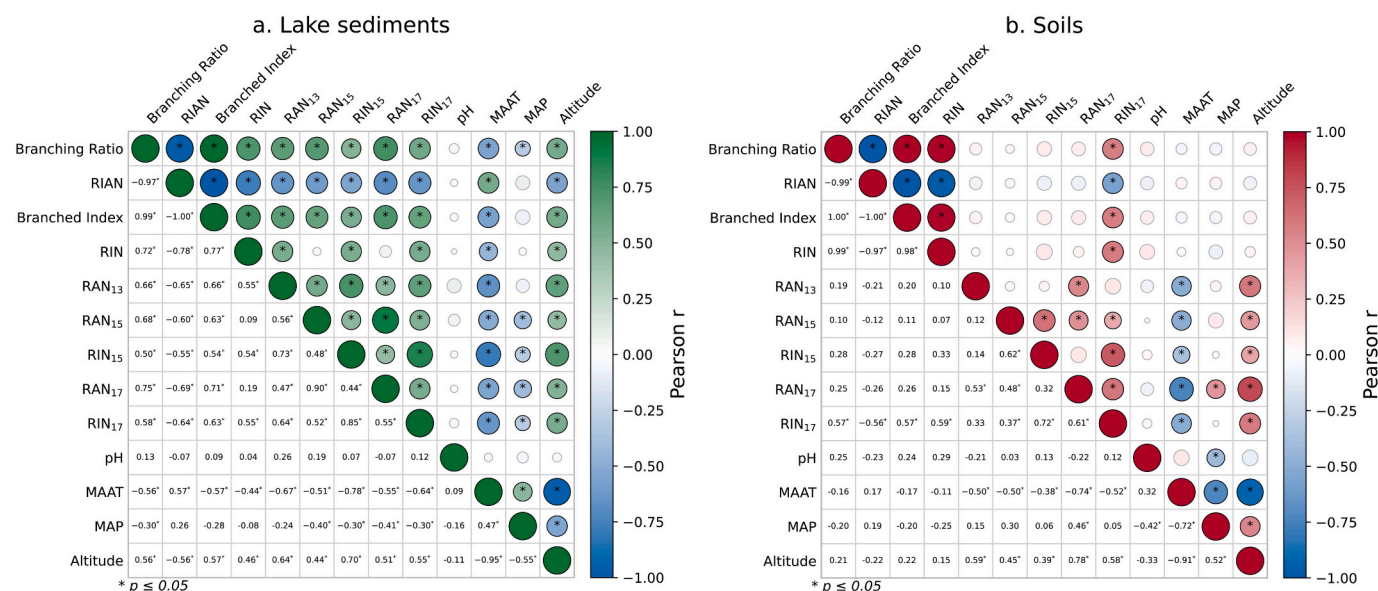


Fig. 6. Heat map showing significant ($p \leq 0.05$) Pearson's correlation coefficients (circles) among the environmental parameters and 3-OH-FA-based indices measured on (a) lake surface sediment samples ($n = 48$, this study), and (b) catchment soil samples ($n = 30$, this study).

lacustrine datasets, including lakes from the eastern United States and China, indicating that the observed brGDGT signatures are characteristic of lacustrine environments influenced by both catchment-derived input and in situ aquatic production. In contrast, soil samples cluster closely with global soil datasets, supporting a predominantly terrestrial origin and showing strong consistency with previous soil-based studies (Fig. 5b).

The methylation pattern is strongly dominated by tetramethylated brGDGTs, whereas hexamethylated compounds occur in relatively low abundances, particularly in the tropical lakes examined here (Fig. 5b). This pattern is likely related to temperature control on bacterial membrane adaptation. Higher temperatures generally favor lower degrees of

methylation, while colder conditions promote the production of more highly methylated brGDGTs to maintain membrane fluidity (Weijers et al., 2007; Martínez-Sosa et al., 2021; Raberg et al., 2021). Accordingly, the relatively low abundance of hexamethylated brGDGTs observed in this study is consistent with the warm climatic conditions of tropical Southeast Asia. Similarly, soils from this study are characterized by relatively low abundances of pentamethylated and hexamethylated compounds compared to datasets from higher-latitude regions. This trend further supports climatic controls on brGDGT methylation in tropical environments, where relatively high temperatures at lower latitudes favor lower degrees of methylation compared to colder, higher-latitude settings (Fig. 5b).

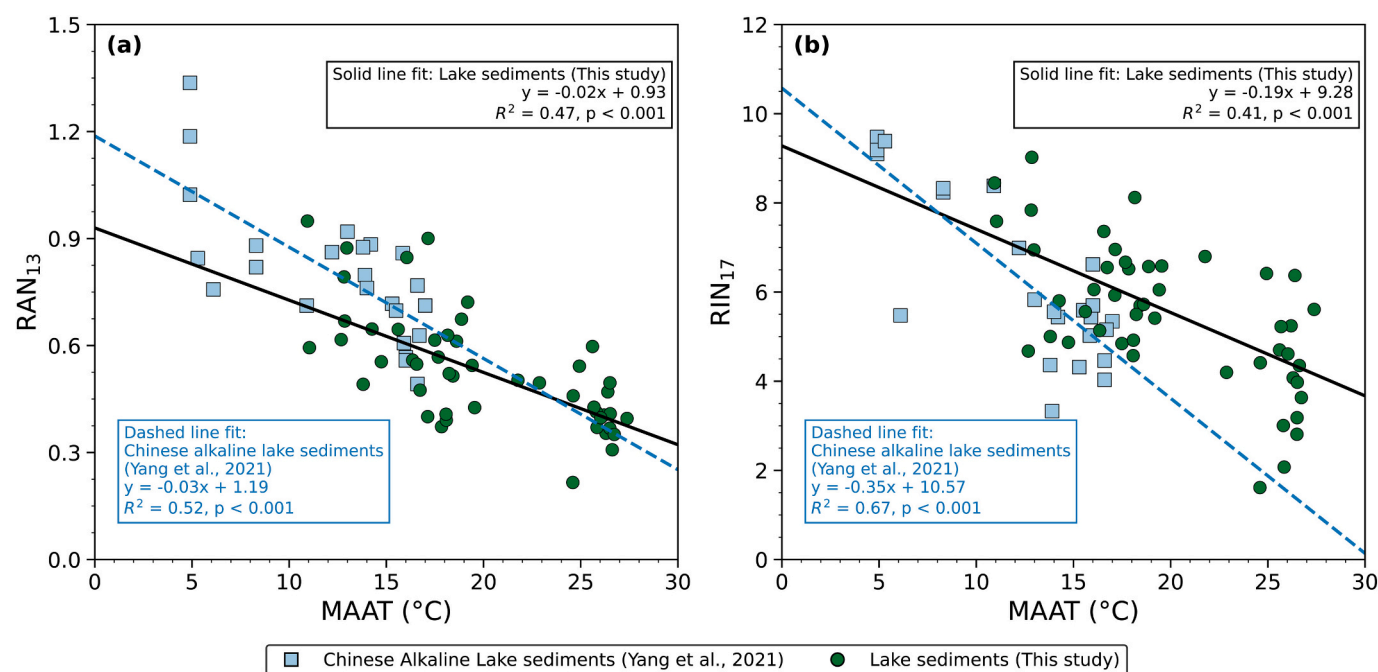


Fig. 7. Scatter plots of (a) RAN₁₃ and (b) RIN₁₇ against mean annual air temperature (MAAT) in lake surface sediment samples in this study (black solid line) and the Chinese alkaline lake dataset (light blue dashed line; Yang et al., 2021). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

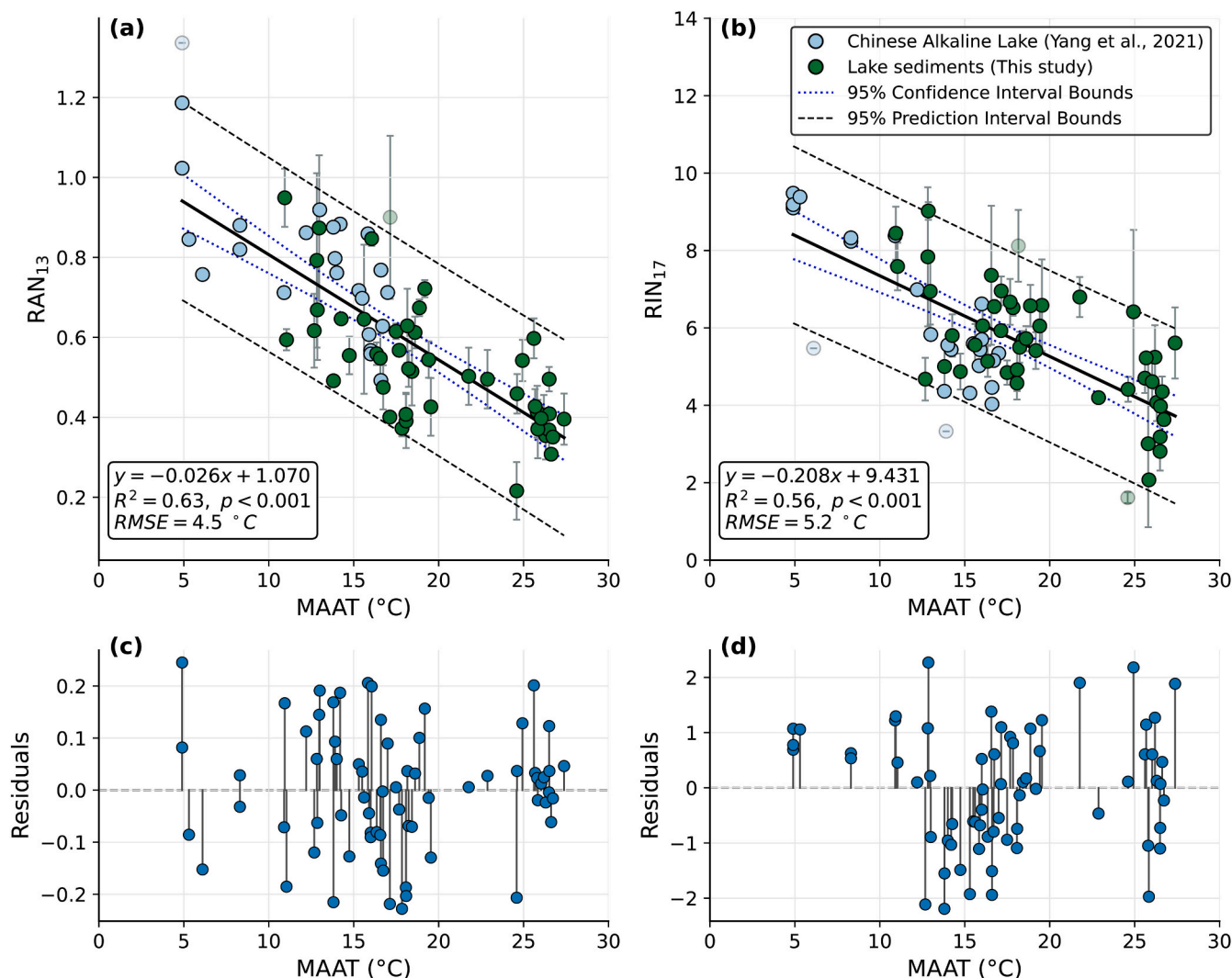


Fig. 8. Scatter plots of (a) RAN₁₃ and (b) RIN₁₇ against mean annual air temperature (MAAT) in lake surface sediment samples combined with the Chinese alkaline lake dataset (Yang et al., 2021). Shown are the 95% confidence interval (blue dashed line) and prediction interval bounds (black dashed line), representing a 95% probability that a new observation and the true function, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.2. Empirical relationships of 3-OH-FA-based indices in catchment soils and lake sediments across Southeast Asia

4.2.1. 3-OH-FA-based temperature indices in lakes

The empirical relationships of temperature indices based on 3-OH-FAs have previously demonstrated significant correlations with environmental parameters in terrestrial and aquatic settings (Wang et al., 2016, 2021; Huguet et al., 2019; Yang et al., 2020, 2021; Véquaud et al., 2021b). In this study, the temperature sensitivity of 3-OH-FA-derived indices is evaluated along a Southeast Asian transect using paired lake sediment and catchment soil samples shown in a heatmap (Fig. 6). As discussed above, 3-OH-FA distributions in lake sediments reflect a combination of in situ aquatic production and partial contributions from surrounding soils, resulting in distinct compositional characteristics. Consequently, the independent development of 3-OH-FA-based temperature indices in soils and lake sediments emphasizes the influence of environmental factors—particularly temperature and pH—on bacterial lipid compositions (Yang et al., 2021). The heatmap analysis of lake sediments (Fig. 6a) shows that the two soil-based temperature indices, RAN₁₅ and RAN₁₇, exhibit moderate correlations with MAAT, with Pearson's r values of -0.51 ($p < 0.05$) and -0.55 ($p < 0.05$), respectively. These relatively moderate relationships are

consistent with the linear regression results obtained for lake sediment samples (RAN₁₅: slope = -0.30 , $R^2 = 0.26$, $p < 0.001$; RAN₁₇: slope = -0.24 , $R^2 = 0.29$, $p < 0.001$; Fig. S1). The results suggest that RAN₁₅ and RAN₁₇ in lake sediments do not reliably reflect catchment MAAT. Furthermore, the distributions of 3-OH-FAs in lake environments are likely influenced by a combination of allochthonous soil-derived input and autochthonous production within the lake. Such in situ production may partially or completely overprint the original soil-derived temperature signal, thereby limiting the applicability of these indices in lacustrine settings, consistent with previous observations by Yang et al. (2021). In addition, MAP also showed relatively weak Pearson's correlations with the temperature-related indices RAN₁₃ and RIN₁₇ in lake sediments ($r = -0.24$, $p < 0.05$) and $r = -0.30$, $p < 0.05$, respectively; Fig. 6a). Although altitude showed moderate correlations with these indices ($r = 0.64$, $p < 0.05$ and $r = 0.55$, $p < 0.05$, respectively; Fig. 6a), the corresponding regression relationships were relatively weak (low R^2), indicating that altitude explained only a small proportion of their variability. Thus, MAP and altitude were not considered further as potential controls on these proxies.

The RAN₁₃ index is defined as the ratio of *anteiso* C₁₃ to *normal* C₁₃ 3-OH-FAs (Eq. (1)). The RAN₁₃ index was initially proposed by Yang et al. (2020) as a sea surface temperature (SST) proxy in a marine setting and

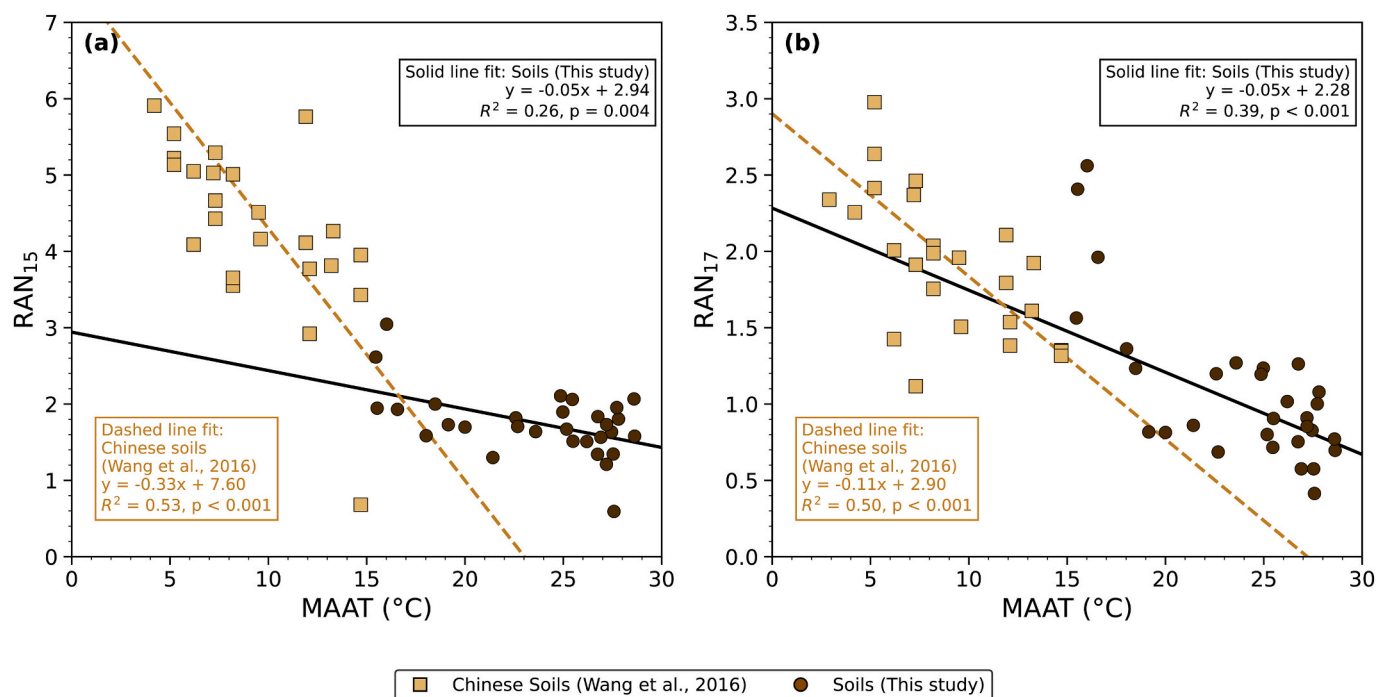


Fig. 9. Scatter plots of (a) RAN_{15} and (b) RAN_{17} against mean annual air temperature (MAAT) in soil samples in this study (black solid line) and the Mt. Shennongjia soil transect dataset (light brown dashed line; Wang et al., 2016). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was evaluated for temperature sensitivity in a lake environment (Yang et al., 2021). Previous works show that the RAN_{13} index exhibits an exponential relationship with MAAT in the South China Sea (slope = -2.11 , $R^2 = 0.92$, $p < 0.05$; Yang et al., 2020) and exhibits a linear regression with MAAT in Chinese alkaline lakes (slope = -0.031 , $R^2 = 0.50$, $p < 0.001$; Yang et al., 2021). In this study, the RAN_{13} index is moderately correlated with MAAT in the Southeast Asian lakes (slope = -0.02 , $R^2 = 0.47$, $p < 0.001$; Fig. 7a). The significant correlation between RAN_{13} with MAAT suggests bacterial membrane lipid composition adapts to temperature, with *anteiso* 3-OH-FAs enhancing membrane fluidity in cooler environments (Wang et al., 2016; Yang et al., 2020, 2021). Similarly, the data from this study are compared with those from a previous investigation of 3-OH-FAs in lake surface sediments from Central China by Yang et al. (2021), as shown in Fig. 7a. The similar linear regression trends observed in both datasets suggest that the RAN_{13} –MAAT calibration may be applicable beyond a regional scale, suggesting its broader potential for temperature calibration in a lake setting. Therefore, combining the two datasets strengthens the correlation between RAN_{13} and MAAT (Fig. 8a), resulting in an improved coefficient of determination (slope = -0.026 , $R^2 = 0.63$, $p < 0.001$). This enhancement allows for the development of an updated empirical relationship of the RAN_{13} index as a temperature calibration (Eq. (15)).

The RIN_{17} index is defined as the ratio of *iso* C_{17} to *normal* C_{17} 3-OH-FAs (Eq. (2)). The RIN_{17} index was proposed by Yang et al. (2021) as a novel 3-OH-FA-based temperature proxy in lake environments. The RIN_{17} index shows a linear regression with MAAT in Chinese alkaline lakes (slope = -0.35 , $R^2 = 0.67$, $p < 0.001$; Yang et al., 2021). In contrast, the RIN_{17} index shows a weaker correlation with MAAT in the Southeast Asian lakes (slope = -0.19 , $R^2 = 0.41$, $p < 0.001$; Fig. 7b), indicating a regional limitation to use as a lake temperature calibration in this region. We attribute this to regional differences in bacterial, depositional, and terrestrial input variability (e.g., Véquaud et al., 2021b; Wang et al., 2021). Although the linear trend observed in this study is less steep compared to the previous study (Fig. 7b), the combined dataset yields a moderate correlation between RIN_{17} and MAAT

across an extended temperature range (slope = -0.208 , $R^2 = 0.56$, $p < 0.001$; Fig. 8b). This suggests that the RIN_{17} index may require site-specific calibration to account for local environmental factors. However, this study provides an updated and broader MAAT calibration for the RIN_{17} index (Eq. (16)).

The linear regression equations derived from the 3-OH-FA-based temperature indices from lake surface sediments versus MAAT are as follows (Fig. 8a and b):

$$MAAT = 41.15 - 38.46 \times RAN_{13} \quad (R^2 = 0.63, p < 0.001, n = 69, RMSE = 4.5^\circ C) \quad (15)$$

$$MAAT = 45.34 - 4.81 \times RIN_{17} \quad (R^2 = 0.56, p < 0.001, n = 69, RMSE = 5.2^\circ C) \quad (16)$$

4.2.2. 3-OH-FA-based temperature indices in soils

The empirical relationships of 3-OH-FA-based temperature proxies with environmental parameters are also evaluated along the Southeast Asian transect of soil samples (Wang et al., 2016). The heatmap reveals that the RAN_{13} and RIN_{17} indices, previously proposed as lake temperature calibrations by Yang et al. (2021), show Pearson's r values of -0.50 ($p < 0.05$) and -0.52 ($p < 0.05$), respectively (Fig. 6b). These relatively moderate relationships are consistent with the linear regression results obtained for soil samples (RAN_{13} : slope = -0.01 , $R^2 = 0.04$, $p = 0.328$; RIN_{17} : slope = -0.36 , $R^2 = 0.25$, $p = 0.005$; Fig. S2), suggesting limited applicability in soil environments. This limitation likely results from differences in bacterial community composition, as in situ aquatic bacteria contribute distinct 3-OH-FA signals not present in soils (Yang et al., 2020, 2021). In addition, MAP showed relatively weak Pearson's correlations with the temperature-related indices RAN_{15} and RAN_{17} in soils ($r = 0.30$, $p < 0.05$ and $r = 0.46$, $p < 0.05$, respectively; Fig. 6b), while altitude showed stronger correlations ($r = 0.45$, $p < 0.05$ and $r = 0.78$, $p < 0.05$, respectively; Fig. 6b), but weak regression relationships (low R^2), indicating that altitude explained little of the observed variability. These results are broadly consistent with Wang et al. (2021), who found no significant effects of MAP and altitude on

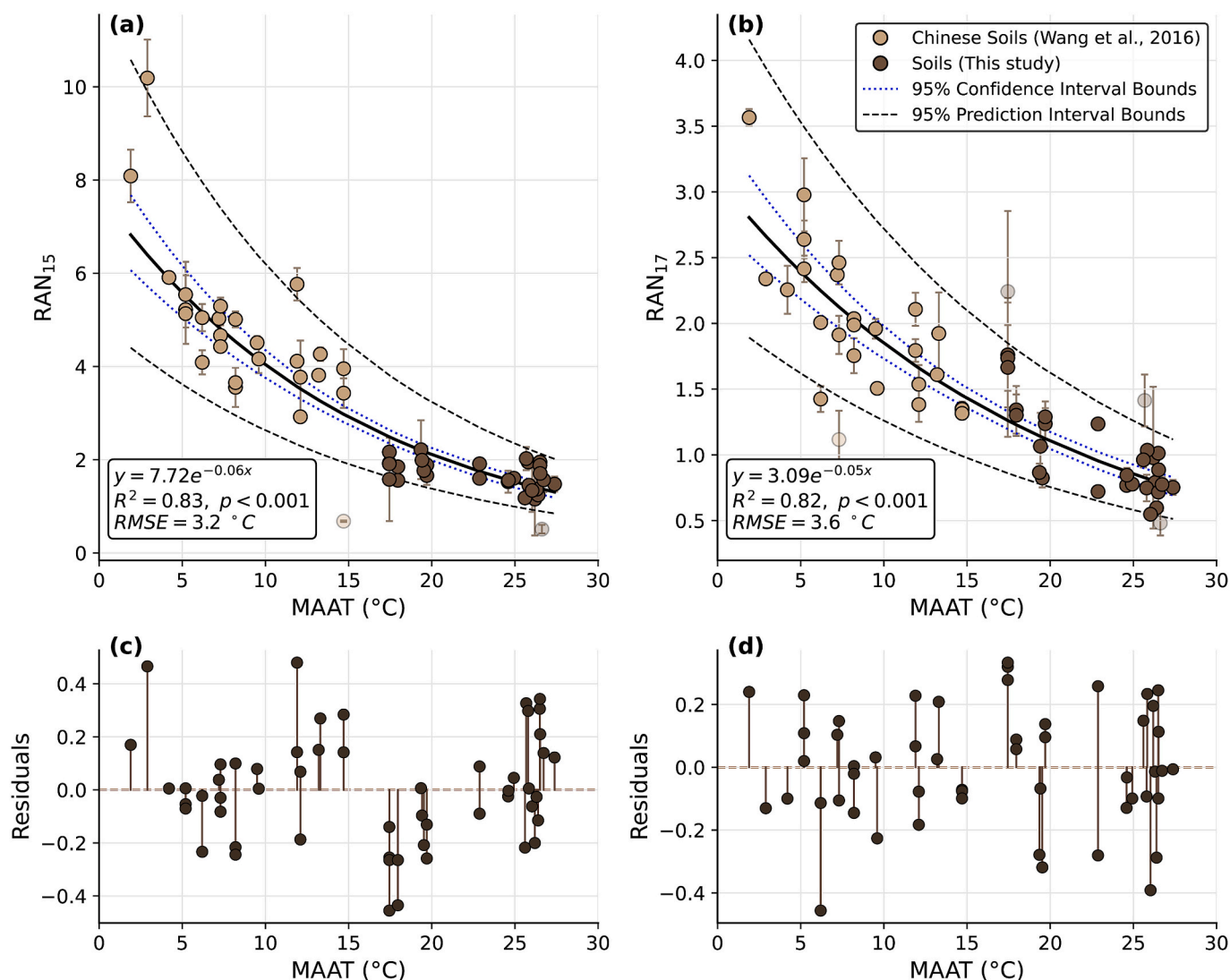


Fig. 10. Scatter plot of (a) RAN_{15} and (b) RAN_{17} against mean annual air temperature (MAAT) in soil samples combined with the Mt. Shennongjia soil transect dataset (Wang et al., 2016). Shown are the 95% confidence interval (blue dashed line) and prediction interval bounds (black dashed line), representing a 95% probability that a new observation and the true function, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3-OH-FA distributions in global soils, suggesting that neither variable is a primary control on these proxies. However, the RAN_{15} index shows a weak correlation with MAAT in the Southeast Asian soils (slope = -0.05 , $R^2 = 0.26$, $p = 0.004$; Fig. 9a; Eq. (3)), contrasting with its performance in other climate regions with slope = -0.33 , $R^2 = 0.53$, $p < 0.001$ (e.g., Wang et al., 2016; Huguet et al., 2019). This reduced sensitivity is likely driven by strong influences from local environmental factors, especially the soil bacterial community, vegetation, and soil organic input (Wang et al., 2021). However, the RAN_{17} index shows slightly higher correlation with MAAT compared to RAN_{15} index in this study (slope = -0.05 , $R^2 = 0.39$, $p < 0.001$; Fig. 9b; Eq. (4)), consistent with its application in global soil calibrations (Wang et al., 2021). Thus, the Southeast Asian soil samples generally fit established global calibration trends for both RAN_{15} and RAN_{17} indices (Fig. S3); however, they exhibit notably flatter regression slopes—particularly in comparison to the Mt. Shennongjia soil transect in Central China (Wang et al., 2016, 2021; Fig. 9a and b). This divergence is attributed to a possible loss of sensitivity in both RAN_{15} and RAN_{17} indices at MAATs exceeding ca. 25°C , where Gram-negative bacteria regulation becomes less responsive under high thermal conditions, likely due to decreased pressure for homeoviscous adaptation (Huguet et al., 2019).

Consequently, exponential regression provides a better fit across broader climatic gradient from integrating data between Mt. Shennongjia and Southeast Asia soils, showing slope = -0.06 , $R^2 = 0.83$, $p < 0.001$ for the RAN_{15} index and slope = -0.05 , $R^2 = 0.82$, $p < 0.001$ for the RAN_{17} index (Fig. 10a and b), indicating a nonlinear temperature response under consistently warm environmental conditions (Eqs. (17) and (18)).

The exponential regression shown in Fig. 10a and b, equations from 3-OH-FA-based temperature indices vs. MAAT in lake sediments were generated as follows:

$$MAAT = 34.00 - 16.67 \ln(RAN_{15}) \quad (R^2 = 0.83, p < 0.001, n = 56, RMSE = 3.2^\circ\text{C}) \quad (17)$$

$$MAAT = 22.57 - 20.00 \ln(RAN_{17}) \quad (R^2 = 0.82, p < 0.001, n = 56, RMSE = 3.6^\circ\text{C}) \quad (18)$$

4.2.3. 3-OH-FA-based pH indices in lakes and soils

The 3-OH-FA-based pH indices (Eqs. (6)–(9)), including the Branching Ratio, RIAN, Branched Index, and RIN, were initially proposed by Wang et al. (2016), who found the relationship between

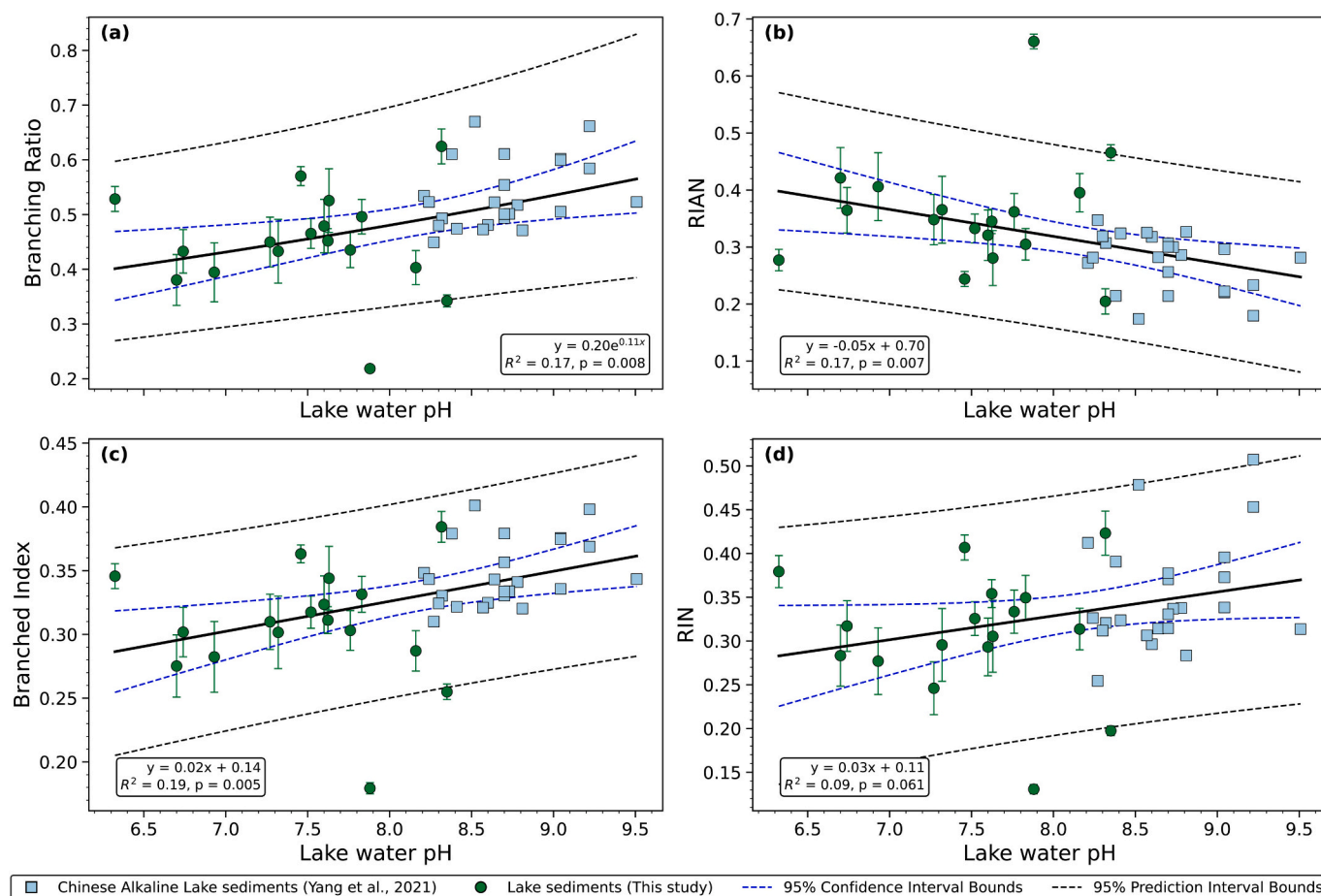


Fig. 11. Scatter plots of 3-OH-FA-based pH indices, (a) the Branching Ratio (BR), (b) RIAN, (c) the Branched Index (BI), and (d) RIN against lake water pH in lake surface sediments combined with the Chinese alkaline lake dataset (Yang et al., 2021). Shown are the 95% confidence interval (blue dashed line) and prediction interval bounds (black dashed line), representing a 95% probability that a new observation and the true function, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

branched-/straight-chained 3-OH-FA ratios and soil pH. Then, the pH indices were applied to soil pH across global soil transects (e.g., Huguet et al., 2019; Wang et al., 2021) and also tested in lake water pH from Chinese alkaline lakes (Yang et al., 2021). The pH indices based on 3-OH-FAs have a linear relationship with soil pH (Wang et al., 2016), but no significant correlation with lake water pH (Yang et al., 2021). In this study, the performance of 3-OH-FA-based pH indices was evaluated across Southeast Asian and Chinese alkaline lake sediments that span a wide range of water pH values (Yang et al., 2021). The results indicate weak or non-significant correlations between these indices and measured lake water pH (Branching Ratio: slope = 0.11, $R^2 = 0.17$, $p = 0.008$; RIAN: slope = -0.05 , $R^2 = 0.17$, $p = 0.007$; Branched Index: slope = 0.02, $R^2 = 0.19$, $p = 0.005$; RIN: slope = 0.03, $R^2 = 0.09$, $p = 0.061$; Fig. 11). Even weaker or non-significant relationships were observed for additional 3-OH-FA-based pH indices in Southeast Asian lake sediments alone (Fig. S4). Several factors likely contribute to this limitation. First, the narrow pH range observed across the study sites (Lake water pH values: 6.3–9.3) reduces the ability to detect meaningful empirical relationships, as previously noted in other pH proxy studies (Huguet et al., 2019; Véquaud et al., 2021b). Second, in situ aquatic bacterial production may obscure the soil pH-derived 3-OH-FA signal (Yang et al., 2021). In lakes, bacterial communities differ both taxonomically and functionally across sites, and these community differences influence lipid biosynthesis pathways that are not strictly driven by lake pH (Yang et al., 2021). Lake pH also interacts with other environmental parameters—such as redox conditions, temperature, and nutrient availability—to influence the relative abundance of branched

versus straight-chained 3-OH-FAs (Huguet et al., 2019). Furthermore, lake water pH can be buffered by carbonate equilibria and hydrological inflows (Wetzel, 2001), decoupling it from the biological processes that regulate 3-OH-FA distributions. The application of 3-OH-FA-based pH indices to aquatic environments appears limited, perhaps due to bacterial dynamics, seasonality, or other environmental factors, influencing lipid distributions. This suggests that regional pH calibrations may exhibit relatively weak correlations, likely due to the limited pH range represented in the current dataset. Expanding the dataset to include sites spanning a broader range of water pH conditions may improve the performance of these indices. Therefore, incorporating samples from environments covering a wide pH gradient will be essential for developing more robust and reliable pH calibrations.

In addition, we also assessed the performance of 3-OH-FA-based pH indices in the Southeast Asian soils combined with the Mt. Shennongjia soil transect in Central China (Wang et al., 2016). The results also reveal weak or inconsistent correlations across the region, indicating limited applicability of these indices for reconstructing soil pH (Branching Ratio: slope = 0.13, $R^2 = 0.24$, $p < 0.001$; RIAN: slope = -0.05 , $R^2 = 0.21$, $p < 0.001$; Branched Index: slope = 0.03, $R^2 = 0.25$, $p < 0.001$; RIN: slope = 0.04, $R^2 = 0.25$, $p < 0.001$; Fig. 12). Similar scatter and weak relationships are also observed in Southeast Asian soils alone (Fig. S5), consistent with previous studies. For example, Huguet et al. (2019) found weak relationships between 3-OH-FA-based pH indices and soil pH. In contrast, the Mt. Shennongjia soil transect in China demonstrates a strong 3-OH-FA-based pH correlation (slope = 0.28, $R^2 = 0.76$, $p < 0.001$), likely due to broader

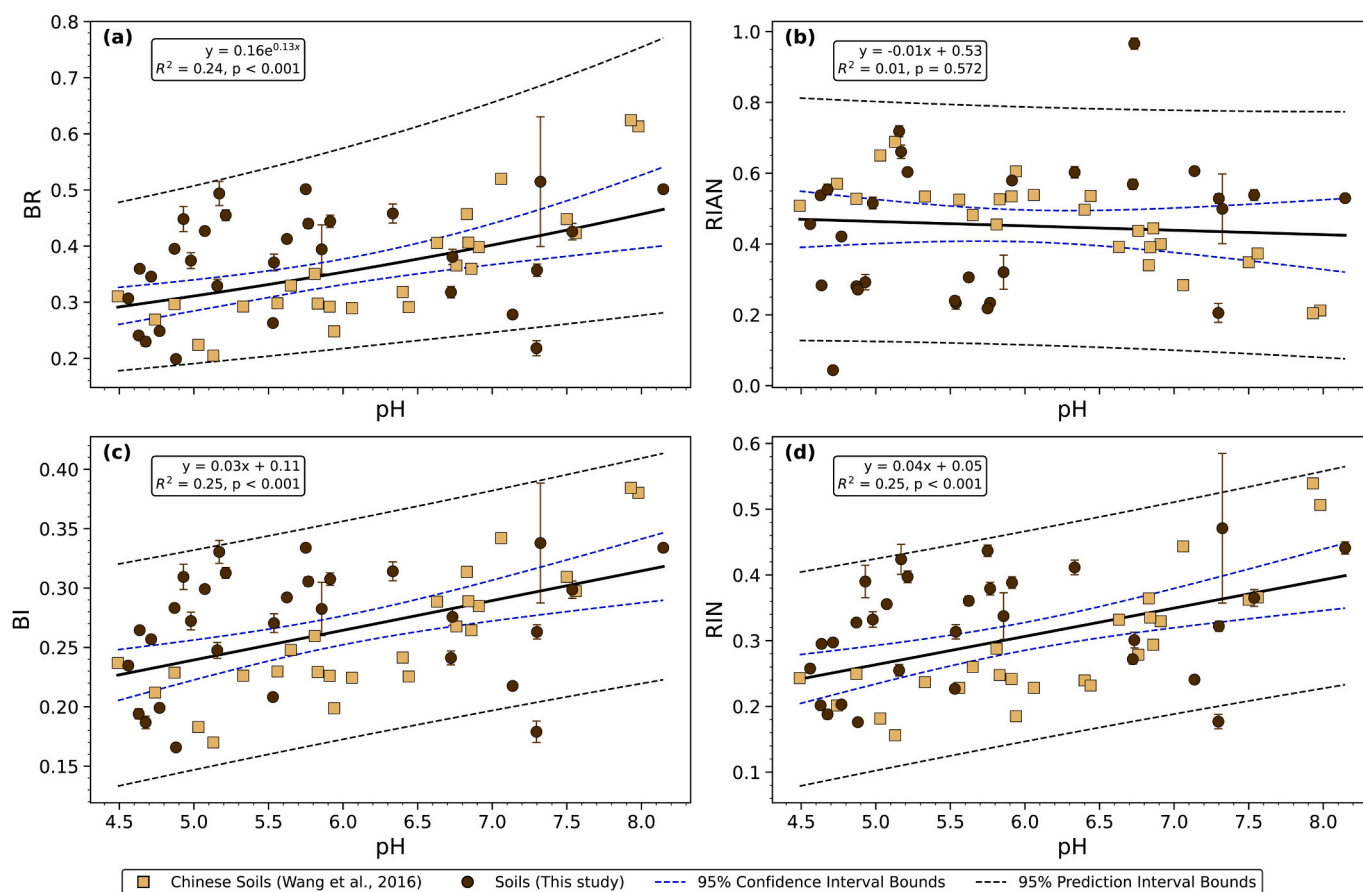


Fig. 12. Scatter plots of 3-OH-FA-based pH indices, (a) the Branching Ratio (BR), (b) RIAN, (c) the Branched Index (BI), and (d) RIN against soil pH in soil samples combined with the Mt. Shennongjia soil transect dataset (Wang et al., 2016). Shown are the 95% confidence interval (blue dashed line) and prediction interval bounds (black dashed line), representing a 95% probability that a new observation and the true function, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

environmental gradients and more distinct bacterial responses (Wang et al., 2016). These weak correlations were attributed to a restricted soil pH range (Soil pH values: 4.6–8.2), which limits the ability to detect robust empirical relationships (Huguet et al., 2019). In addition, Véquaud et al. (2021b) noted that overlapping environmental factors—such as temperature, moisture, and bacterial community composition—can obscure pH-driven lipid patterns in soils. Thus, these findings suggest that although 3-OH-FA-based indices show potential in certain environments, their applicability for pH reconstruction in Southeast Asian soils remains limited due to the relatively narrow pH range represented in the dataset and uncertainties regarding bacterial lipid sources. Consequently, site-specific investigations are needed to better characterize pH variability, constrain the origins of 3-OH-FAs, and improve the reliability and robustness of 3-OH-FA-based pH proxies in soils.

Overall, the findings indicate that the reliability of 3-OH-FA-based pH proxies varies among study sites and datasets. While improved relationships were observed when datasets encompassing broader pH gradients were included, such as Chinese alkaline lakes and the Mt. Shennongjia soil transect, proxy performance remained inconsistent across the investigated environments. These results suggest that the applicability of 3-OH-FA-based pH calibrations is not solely determined by regional environmental settings, but may also be influenced by variations in bacterial community composition and lipid sources. Therefore, future studies should prioritize site-specific investigations that incorporate a wider range of pH conditions and better constrain the microbial sources of 3-OH-FAs through bacterial community analyses. However, in regions with limited natural pH variability, increasing

sample numbers may not improve pH proxy performance, as the narrow pH range constrains calibration potential. Thus, 3-OH-FA-based pH proxies may have lower utility than temperature proxies in such settings. Such approaches will be essential for evaluating the conditions under which 3-OH-FA-based pH proxies perform reliably and for developing more robust and transferable pH calibrations.

4.3. Development of a novel lake temperature index based on 3-OH-FAs for Southeast Asia

Among all tested indices, we find that RIN_{15} exhibits the significant correlation with MAAT in the studied lake sediments (slope = -0.19 , $R^2 = 0.61$, $p < 0.001$; Eq. (5), Fig. 13), outperforming other 3-OH-FA-based indices in the region. This finding suggests that RIN_{15} captures region-specific bacterial adaptations in membrane lipid composition, potentially driven by thermal variability in tropical lacustrine environments. Previous studies have linked isobranched 3-OH-FAs, particularly $isoC_{17}$, to bacterial membrane homeostasis under alkaline lakes and variable environmental conditions (Kaneda, 1991; Zhang and Rock, 2008). However, our results indicate that in Southeast Asian lakes, shorter-chain homologs such as $isoC_{15}$ exhibit greater temperature sensitivity. In this study, we compared our results with those from Chinese alkaline lakes reported by and found that although the linear regression trends appear similar, the correlation between RIN_{15} and MAAT in the Chinese dataset is significantly weaker than in our study (slope = -0.12 , $R^2 = 0.18$, $p < 0.037$; Fig. 13). Thus, this index was not promoted by the authors and suggests that RIN_{15} may not be applicable in Chinese alkaline lakes (Yang et al., 2021). Additionally, the RIN_{17}

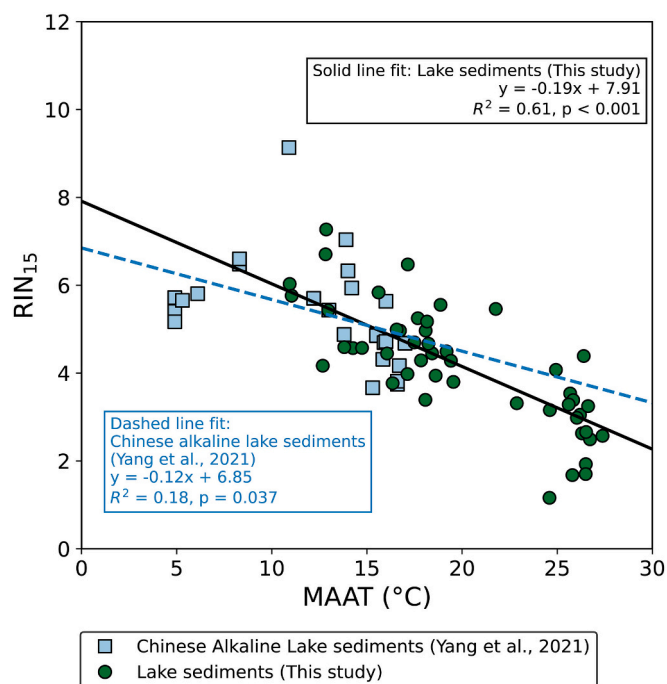


Fig. 13. Scatter plots of RIN_{15} against mean annual air temperature (MAAT) in lake surface sediment samples from this study (black solid line) and the Chinese alkaline lake dataset (light blue dashed line; Yang et al., 2021). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

index—previously identified as temperature-sensitive in Chinese alkaline lakes—exhibited a weaker correlation with MAAT in our Southeast Asian dataset (Fig. 7b). This discrepancy is likely due to differences in lake water chemistry, bacterial community composition, and external organic inputs, all of which can influence lipid distributions. Based on these findings, we propose a novel 3-OH-FA-based temperature calibration using the RIN_{15} index, which shows promise for application across a broader range of lake environments (Eq. (19)).

The linear regression shown in Fig. 14, equations from RIN_{15} vs. MAAT in lake sediments was created as follows:

$$MAAT = 45.00 - 6.17 \times RIN_{15} \quad (R^2 = 0.63, p < 0.001, n = 69, RMSE = 4.6^\circ C) \quad (19)$$

Our findings highlighted the need for regional calibrations of 3-OH-FA-based temperature proxies, particularly for indices like RIN_{15} and RIN_{17} , which appear more responsive to local hydroclimatic conditions than to global trends. In contrast, the RAN_{13} index, linked to in situ bacterial production within the lake sediments, shows consistent performance across diverse lake environments (Yang et al., 2021). In addition to the calibration results, the RAN_{15} and RAN_{17} indices appear to be the most suitable proxies for reconstructing temperature in soils, whereas the RAN_{13} and RIN_{15} indices show the strongest relationships with MAAT in Southeast Asian lake sediments. The RIN_{17} index, which was originally developed from alkaline lakes in China, also exhibits a statistically significant relationship with MAAT in our lake dataset. However, its performance is reduced in Southeast Asian lakes, where greater data scatter results in a weaker correlation compared to RAN_{13} and RIN_{15} . Consequently, RIN_{15} emerges as a promising lake-based temperature proxy for Southeast Asia, while RAN_{13} appears to capture a more universal temperature signal, exhibiting broader applicability and greater transferability across diverse lacustrine environments than region-specific calibrations.

4.4. Regional brGDGT calibrations for soil and lake environments in Southeast Asia

4.4.1. brGDGT-based temperature indices in Southeast Asian lakes

The GDGT-based indices were evaluated along our Southeast Asian transect to complement the 3-OH-FA-based indices (Fig. 3e–i and Supplementary Data). The 5-methyl branched GDGT temperature index (MBT'_{5ME}) shows a strong relationship with MAAT (slope = 0.026, $R^2 = 0.71$, $p < 0.001$; Fig. 15a). When combined with the global lake dataset of Raberg et al. (2022), the Southeast Asian sites closely follow the established global trend, yielding a comparable and robust calibration (slope = 0.020, $R^2 = 0.74$, $p < 0.001$; Fig. 15b). This agreement indicates that the study sites are consistent with the global lake calibration and supports the applicability of MBT'_{5ME} as a temperature proxy across a broad range of lacustrine environments, extending beyond regional-scale settings. This result is further supported by Martínez-Sosa et al. (2021) that the MBT'_{5ME} index correlates even more strongly with the mean temperature of months above freezing (MAF; $R^2 = 0.82$, $p < 0.001$), suggesting high performance in the warm, seasonally stratified lakes investigated in this study. In contrast, the MBT'_{6ME} index displays only moderate predictive ability for MAAT in Southeast Asian lakes alone (slope = 0.017, $R^2 = 0.39$, $p < 0.001$; Fig. S6a); however, when combined with datasets from Chinese alkaline lakes, the MBT'_{6ME} index-MAAT correlation significantly increases (slope = 0.21, $R^2 = 0.60$, $p < 0.001$; Fig. S6b). Dang et al. (2018) demonstrated that the inclusion of 6-methyl isomers improves temperature sensitivity in alkaline lake environments. However, the additional variability associated with 6-methyl compounds may complicate their calibration and reduce the accuracy of temperature reconstructions (Véquaud et al., 2021b). This reduced performance is likely attributed to seasonal production patterns that obscure annual temperature signals, especially in colder lake settings (Yang et al., 2021). Furthermore, these findings support the exclusion of 6-methyl isomers in brGDGT-based temperature reconstructions to enhance proxy sensitivity and resolution in tropical lakes (Huguet et al., 2019). Thus, we proposed the first regional temperature calibration using the MBT'_{5ME} index in Southeast Asian lakes (Eq. (20)).

The linear regression shown in Fig. 15a, equations from MBT'_{5ME} vs. MAAT in lake sediments were created as follows:

$$MAAT = 38.46 \times MBT'_{5ME} - 7.31 \quad (R^2 = 0.71, p < 0.001, n = 46, RMSE = 3.3^\circ C) \quad (20)$$

4.4.2. BrGDGT-based temperature indices in Southeast Asian soils

The performance of brGDGT temperature indices in Southeast Asian catchment soils is also evaluated (Fig. 3e–i and Supplementary Data). While the MBT'_{5ME} index exhibits some degree of correlation with MAAT, the relationship is comparatively weaker and more variable (slope = 0.013, $R^2 = 0.28$, $p < 0.01$; Fig. 15c), likely reflecting the heterogeneity of soil environments and the influence of diverse bacterial community compositions on GDGT production (De Jonge et al., 2019). In addition, the MBT'_{6ME} index is also evaluated in this study, showing a low correlation with MAAT (slope = 0.004, $R^2 = 0.07$, $p = 0.196$; Fig. S7). However, when our dataset is combined with the global soil dataset compiled by Raberg et al. (2022)—which includes multiple soil transects (e.g., Wang et al., 2016; Huguet et al., 2019)—the MBT'_{5ME} -MAAT correlation improves significantly (slope = 0.019, $R^2 = 0.63$, $p < 0.001$; Fig. 15d). This enhanced correlation suggests that our Southeast Asian data align well with global trends, although the lower correlation observed within our dataset alone may result from limited sample numbers and the specific regional monsoonal climate. Thus, while brGDGT indices remain promising tools for palaeotemperature reconstructions in soils, their application in monsoon-influenced tropical regions should be approached with caution, as pronounced monsoon-driven seasonality in temperature may influence microbial communities and the environmental controls on brGDGT

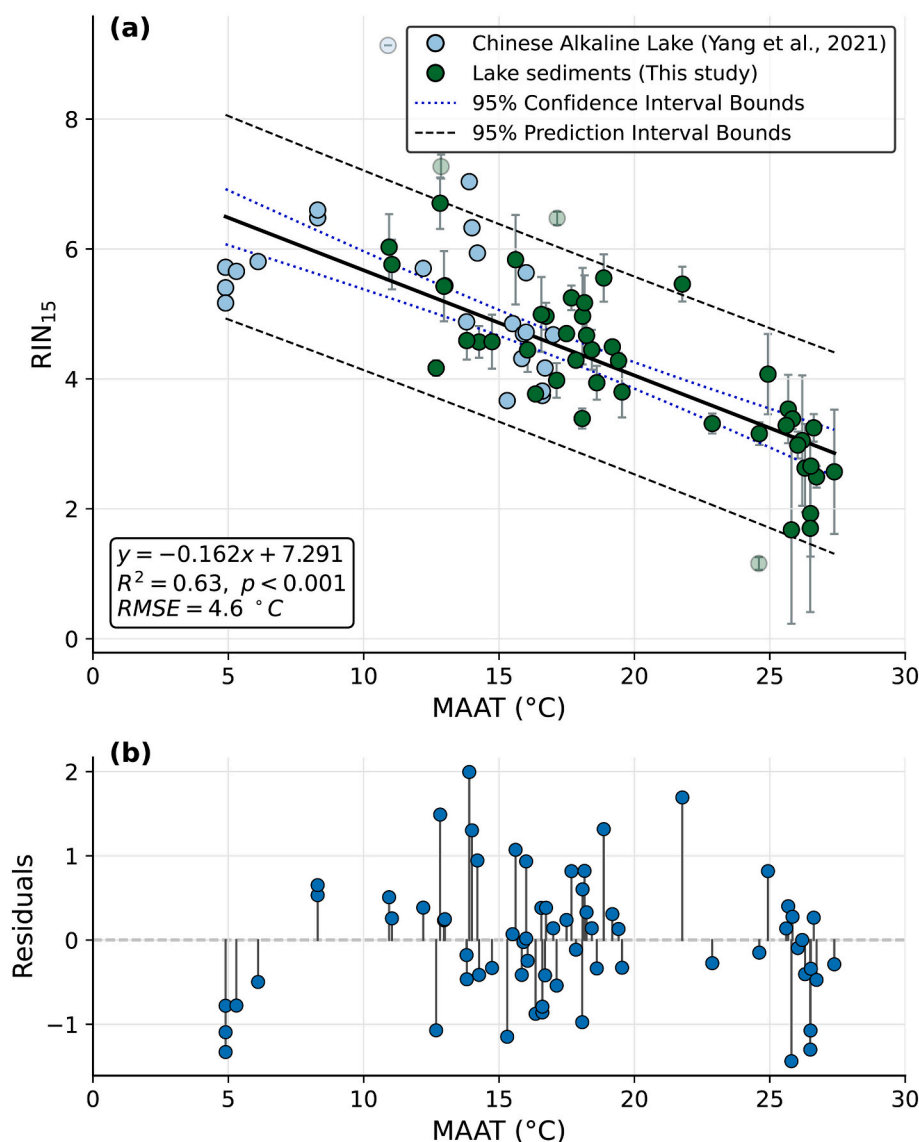


Fig. 14. Scatter plots of RIN_{15} against mean annual air temperature (MAAT) in lake surface sediment samples combined with the Chinese alkaline lake dataset (Yang et al., 2021). Shown are the 95% confidence interval (blue dashed line) and prediction interval bounds (black dashed line), representing a 95% probability that a new observation and the true function, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

production, potentially introducing additional variability into the relationship between brGDGT indices and palaeotemperature. Site-specific or environment-specific calibrations are necessary to account for local variability and improve proxy reliability (Naafs et al., 2017b).

4.4.3. BrGDGT-based pH indices in Southeast Asian lakes and soils

The potential of GDGT-derived pH indices is assessed across the Southeast Asian lakes and soils (Fig. 3e–i and Supplementary Data). The CBT' and IR_{6ME} indices have been proposed for soil and lake water pH calibrations in several previous studies (e.g., Weijers et al., 2007; De Jonge et al., 2014b). In the combined global lake dataset from Yang et al. (2021) and Raberg et al. (2022), the brGDGT-based pH proxy IR_{6ME} shows a moderate, significant relationship with lake water pH (slope = 0.113, $R^2 = 0.41$, $p < 0.001$; Fig. 16a), and Southeast Asian lakes generally align with this global trend. In contrast, the CBT' index exhibits greater scatter and a weaker relationship (slope = -0.217 , $R^2 = 0.30$, $p < 0.001$; Fig. 16b). While both indices correlate poorly when isolating the Southeast Asian data (Fig. S8), their performance improves within the global compilation. This discrepancy highlights the

restricted pH range of the Southeast Asian lakes (pH 6.3–9.3) relative to the broader global gradient (pH 4–10), which provides the statistical power needed to resolve robust empirical trends. However, the deviation of certain Southeast Asian samples suggests that non-pH factors—such as variations in bacterial communities, temperature, redox conditions, and nutrient availability—strongly influence tropical brGDGT distributions (Huguet et al., 2019). Furthermore, catchment hydrology and carbonate buffering can decouple measured lake pH from actual microbial biosynthesis (Wetzel, 2001). These regional complexities suggest that pH alone cannot fully explain brGDGT variability in tropical lakes.

Similarly, soil IR_{6ME} shows expected correlations with pH (slope = 0.183, $R^2 = 0.76$, $p < 0.001$; Fig. 16c). In contrast, CBT' falls outside the global trend obtained from Raberg et al. (2022) with (slope = -0.487 , $R^2 = 0.64$, $p < 0.001$; Fig. 16d). As shown in Fig. S8, these weak relationships are isolated to the Southeast Asian soils, largely due to a restricted pH range (4.6–8.2) that limits statistical power alongside inherent soil heterogeneity (Huguet et al., 2019). Furthermore, overlapping environmental variables—such as temperature,

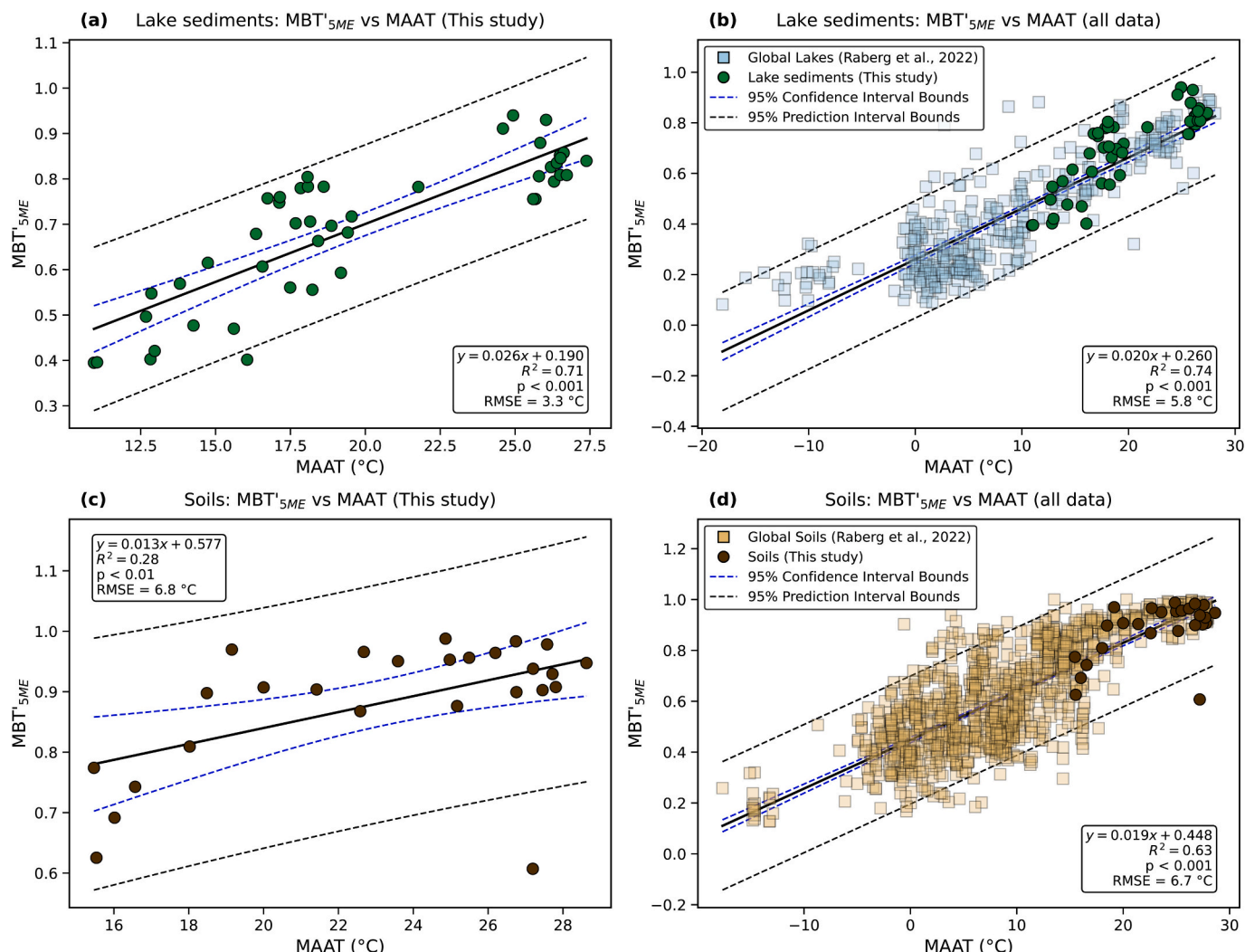


Fig. 15. Scatter plots of (a) MBT'_{5ME} against mean annual air temperature (MAAT) in lake surface sediment samples in this study, (b) MBT'_{5ME} against MAAT in the global lake dataset (Raberg et al., 2022), (c) MBT'_{5ME} against MAAT in soil samples in this study, (d) MBT'_{5ME} against MAAT in the global soil dataset (Raberg et al., 2022). Shown are the 95% confidence interval (blue dashed line) and prediction interval bounds (black dashed line), representing a 95% probability that a new observation and the true function, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

moisture, and bacterial community composition—can obscure pH-driven lipid patterns (Véquaud et al., 2021a, 2021b). This interpretation aligns with Naafs et al. (2017b), who attributed brGDGT methylation patterns to homeoviscous membrane adaptations that become primarily temperature-driven when other environmental variables are minimized. Consequently, expanding regional datasets to cover a broader pH range and incorporating microbial ecological information will be essential for developing robust lake- and soil-specific calibrations. By comparison, the 3-OH-FA-based indices examined here show even weaker correlations with environmental pH, likely reflecting the limited pH range represented in the current dataset rather than an inherent insensitivity.

In conclusion, this study supports the use of MBT'_{5ME} as a robust and reliable temperature proxy for both regional and global palaeoclimate reconstructions, particularly in lake environments. When used in combination with 3-OH-FA-derived temperature indices, these proxies provide complementary insights into environmental conditions across tropical monsoon-dominated regions. For pH calibrations, the application of GDGT-derived indices such as CBT' and IR_{6ME} indices shows promise, especially when broader environmental gradients—such as those encompassing global soils and neutral to alkaline global

lakes—are incorporated into calibration datasets. These indices may outperform 3-OH-FA-based pH proxies, which exhibit limited sensitivity to pH variations in tropical settings and currently lack a well-established global calibration trend. To enhance the precision and broader applicability of biomarker-based palaeoclimate reconstructions, future research should focus on resolving the bacterial sources of lipid biomarkers, understanding how lipids adapt to environmental conditions, and developing integrated multi-proxy approaches that combine the strengths of brGDGTs, 3-OH-FAs, and pH-sensitive indices such as the Branching Ratio, RIN index, CBT' index, and IR_{6ME} index.

More broadly we demonstrate that novel 3-OH-FA-based proxies for temperature and pH continue to have strong potential for application across a wide range of terrestrial and aquatic archives. Because Gram-negative bacteria are ubiquitous, 3-OH-FAs can be found and are preserved in a variety of environments including soils, lake sediments, speleothems (Wang et al., 2018), and marginal marine settings (Yang et al., 2020). This may enable cross-archive comparisons that are particularly valuable for linking terrestrial and marine climate dynamics in monsoon-influenced regions.

Beyond proxy calibration, 3-OH-FAs are amenable to compound-specific isotopic analyses and future work should focus on this area,

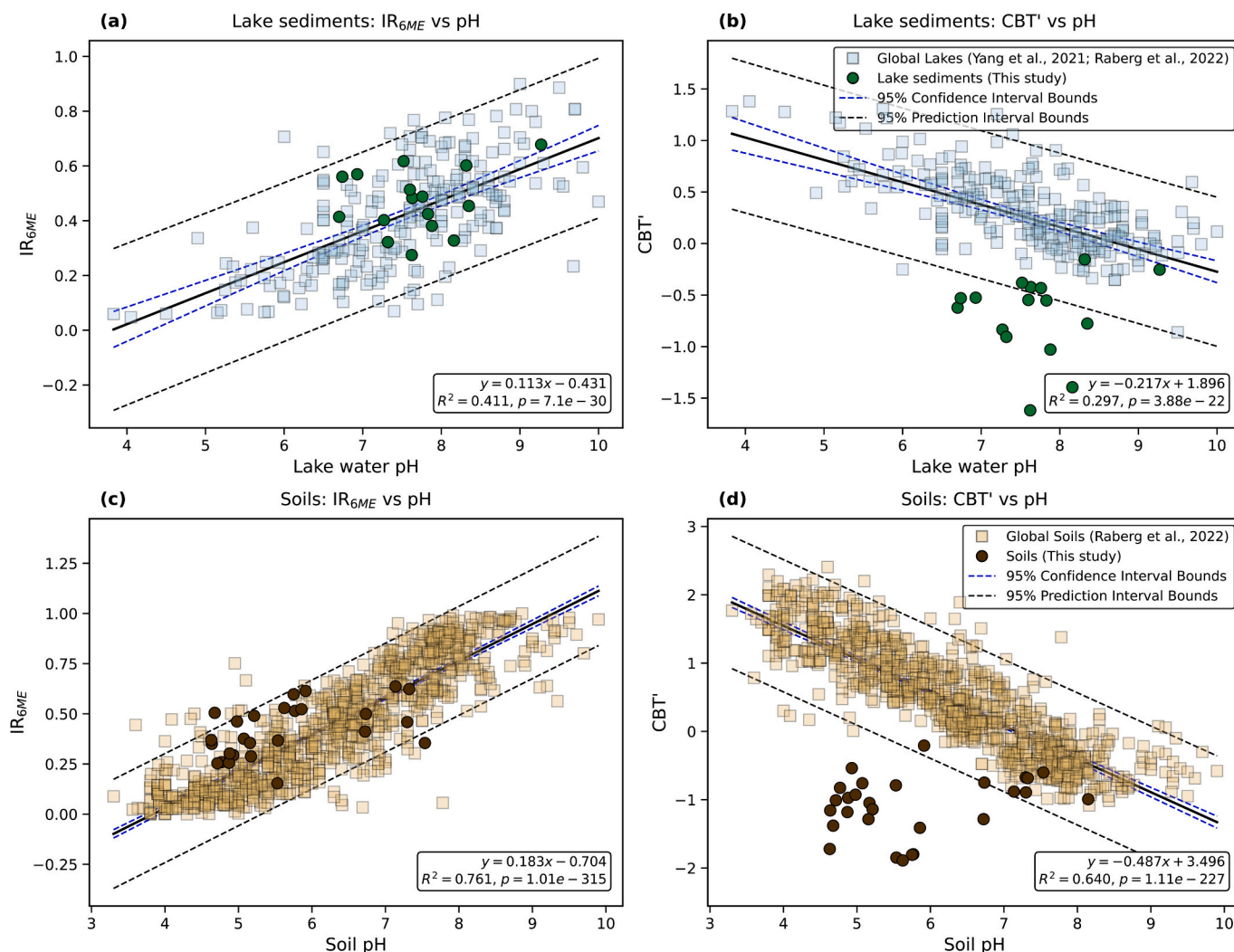


Fig. 16. Scatter plots of (a) IR_{6ME} and (b) CBT' against lake water pH from lake sediment samples in this study combined with the global lake dataset (Yang et al., 2021; Raberg et al., 2022), (c) IR_{6ME} and (d) CBT' against soil pH from soil samples in this study combined with the global soil dataset (Raberg et al., 2022). Shown are the 95% confidence interval (blue dashed line) and prediction interval bounds (black dashed line), representing a 95% probability that a new observation and the true function, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

potentially offering additional constraints on environmental processes such as hydrology (δ^2H) and carbon cycling ($\delta^{13}C$). Their bacterial origin also provides opportunities to integrate palaeoenvironmental reconstructions with modern process-based studies of bacterial ecology and biogeochemistry. Together, these characteristics highlight the considerable further potential of 3-OH-FAs as integrative environmental biomarkers in monsoonal systems.

5. Conclusions

This study investigates 3-OH-FA and brGDGT distributions in lake sediments and soils across Southeast Asia, highlighting distinct bacterial contributions and lipid distributions influenced by in situ production and catchment soil-derived inputs. Among the 3-OH-FA indices, the RAN_{13} index proves to be a promising proxy for lake-based temperature reconstructions, showing consistent correlations with MAAT at both regional and global scales. The RIN_{15} index outperforms the RIN_{17} index in this tropical lake setting, suggesting its stronger suitability for regional lake calibrations. In soils, the RAN_{15} and RAN_{17} indices display a robust global correlation with MAAT. The exponential regression is proposed for these soil-based temperature indices to account for a decline in temperature sensitivity above $\sim 25^\circ C$. For brGDGTs, the

MBT'_{5ME} index exhibits strong correlations with MAAT, confirming its suitability as the most reliable brGDGT-based temperature proxy in both lake and soil environments. In contrast, the MBT'_{6ME} index exhibits weaker correlations, likely due to seasonal biases. Furthermore, combined applications of 3-OH-FA- and brGDGT-based temperature proxies enhance the robustness of empirical calibrations and extend their potential for reconstructing temperature variations over geological time-scales. Although the use of 3-OH-FAs remains relatively limited, particularly in lake environments, their promising temperature sensitivity suggests they may serve as valuable complementary proxies to brGDGTs when appropriately calibrated. Regarding pH calibrations, 3-OH-FA-derived indices generally show weak correlations with lake water and soil pH, whereas brGDGT-derived indices such as CBT' and IR_{6ME} exhibit moderate and more consistent pH sensitivity. Notably, stronger correlations are observed when the pH range is expanded, suggesting that the limited variability in environmental pH may obscure underlying relationships. These findings highlight the importance of regionally constrained and site-specific calibrations, alongside further investigation into bacterial community composition and environmental controls. Such efforts are essential to improve the accuracy and reliability of lipid-based hydroclimatic proxies, particularly in tropical and monsoon-influenced regions.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.orggeochem.2026.105320>.

CRediT authorship contribution statement

Patthapong Chaiseanwang: Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kweku Afrifa Yamoah:** Writing – review & editing, Supervision, Resources, Methodology, Investigation. **Amy Thorpe:** Software, Resources, Methodology, Formal analysis, Data curation. **Mengjing Li:** Software, Methodology, Formal analysis, Data curation. **Lin Chen:** Software, Methodology, Formal analysis, Data curation. **Aifeng Zhou:** Supervision, Resources, Methodology, Funding acquisition. **Huiling Sun:** Supervision, Resources, Investigation. **Matthew Allison:** Software, Resources, Methodology. **Akkaneewut Jirapinyakul:** Supervision, Resources, Investigation. **Quốc Đỗ-Trọng:** Resources. **Vasile Ersek:** Writing – review & editing, Supervision. **Michael Griffiths:** Writing – review & editing, Supervision. **Sarah Greene:** Writing – review & editing, Supervision. **James Bendle:** Writing – review & editing, Validation, Supervision, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Acknowledgements

We thank the reviewers, Dr. Ronnakrit Rattanasriampaipong and the anonymous reviewer, for their constructive comments, which helped improve the quality of this manuscript. This work was supported by funding from the Leverhulme Trust (UK; RPG-2018- 110), the Japan–UK Bilateral Joint Research Project of the Japan Society for the Promotion of Science (JSPS; Grant No. JPJSBP120235701), The Royal Society International Exchanges Cost Share (JSPS) IEC\R3\223007, Project No. 1004029), and The Great Britain Sasakawa Foundation (Grant No. 6509).

Data availability

Data will be made available on request.

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