



**Enhanced production and methylation of bacterial branched tetraether lipids
responding to water content-mediated redox changes in Tibetan Plateau
permafrost soils**

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30 **Abstract**

31 Branched glycerol dialkyl glycerol tetraethers (branched GDGTs) are bacterial
32 membrane lipids widely used as biomarkers for terrestrial and marine
33 paleoenvironmental reconstruction. Sparsely branched and overly branched GDGTs
34 (sb- and ob-GDGTs) are predominantly detected in anaerobic environments and have
35 been proposed as redox proxies in marine settings. However, their distributions,
36 environmental controls, and biological sources in terrestrial environments remain
37 poorly understood. Here, we investigated the distributions of sb/br/ob-GDGTs, together
38 with bacterial community composition in two permafrost peatland soil profiles from
39 the Tibetan Plateau. Our results demonstrate that elevated soil water content (SWC)
40 influences branched GDGT composition by creating anaerobic conditions that promote
41 the production and methylation of sb/br/ob-GDGTs. Co-occurrence network analyses
42 revealed strong positive correlations between the relative abundances of sb/br/ob-
43 GDGTs and anaerobic bacterial communities, including taxa affiliated with
44 *Proteobacteria*, *Caldithrix*, *Nitrospirae*, *Spirochaetes* and *Chloroflexi*. These findings
45 suggest that oxygen-limited conditions may reshape sb/br/ob-GDGT-producing
46 bacterial communities in permafrost soils, thereby promoting the accumulation of
47 sb/br/ob-GDGTs. Furthermore, we demonstrate that the methylation index of ob- and
48 br-GDGTs ($MI_{ob/br}$) serves as a sensitive indicator for soil redox dynamics and may be
49 applied to reconstruct paleohydrological variability, thereby aiding assessments of
50 permafrost carbon-cycle feedbacks to climate warming.

51

52 **Keywords:** sb/ob-GDGTs, methylation, soil water content, anaerobic bacteria, Tibetan
53 Plateau

54



55 1. Introduction

56 Microbial membrane lipids are essential structural components that maintain
57 cellular homeostasis and enhance resistance to environmental stressors (Beney &
58 Gervais, 2001; Guan et al., 2017; Zhang & Rock, 2008). Among these lipids, branched
59 glycerol dialkyl glycerol tetraethers (branched GDGTs) are unique membrane-spanning
60 biomarkers synthesized by certain bacterial groups (Sinninghe Damsté et al., 2011;
61 Weijers et al., 2007). Structurally, branched GDGTs comprise a diverse group of
62 tetraether lipids containing branched alkyl chains, among which the canonical branched
63 GDGTs (hereafter referred to as br-GDGTs) are composed of two 13,16-
64 dimethyloctacosane alkyl chains bearing 0–2 additional methyl substituents and 0–2
65 cyclopentyl moieties. The additional methyl substituents can occur at the α and/or ω -5,
66 6, or 7 positions along the alkyl chains, resulting in C5-, C6-, and C7-methylated br-
67 GDGTs (De Jonge et al., 2013; De Jonge et al., 2014a; Ding et al., 2016; Sinninghe
68 Damsté et al., 2000). Br-GDGTs are ubiquitously detected across diverse environments,
69 including soils, lakes, marine water column and sediments (De Jonge et al., 2014a; De
70 Jonge et al., 2014b; Ding et al., 2016; Sinninghe Damsté et al., 2011; Weijers et al.,
71 2007). Their distributions are strongly correlated with environmental factors such as
72 temperature and soil pH, forming the basis of widely used paleoclimate proxies,
73 including the methylation index of branched tetraethers (MBT), the cyclization ratio of
74 branched tetraethers (CBT), and the isomer ratio (IR) (Dang et al., 2016; De Jonge et
75 al., 2014b; De Jonge et al., 2015; Pei et al., 2021; Peterse et al., 2012; Wang et al., 2021;
76 Wang et al., 2017; Wang et al., 2024; Weijers et al., 2007).

77 Despite their widespread application, the biological sources and ecological
78 controls of br-GDGTs remain incompletely constrained. Previous studies have
79 identified several aerobic Acidobacterial species as producers of specific br-GDGTs
80 (Chen et al., 2022; Halamka et al., 2021; Halamka et al., 2023; Sinninghe Damsté et al.,
81 2018; Sinninghe Damsté et al., 2011; Sinninghe Damsté et al., 2014), yet multiple
82 bacterial phyla other than *Acidobacteria* are indicated as potential producers for br-
83 GDGTs in diverse environments (Tang et al., 2024; Weber et al., 2018; Zeng et al.,



84 2023). Moreover, environmentally driven shift in microbial community composition is
85 also suggested to alter br-GDGT distributions, thereby affecting the reliability of br-
86 GDGT-based proxies (Chen et al., 2024; De Jonge et al., 2019; Liang et al., 2026; Liang
87 et al., 2023; Martínez-Sosa et al., 2026; Tang et al., 2024).

88 On the other hand, recent discoveries have expanded the structural diversity of
89 branched GDGTs based on their degree of methylation, including the identification of
90 overly branched GDGTs (ob-GDGTs) and sparsely branched GDGTs (sb-GDGTs) (Liu
91 et al., 2012). These compounds are closely associated with oxygen-depleted marine
92 environments and have been proposed as indicators of O₂ depletion (Liu et al., 2014;
93 Xie et al., 2014; Zeng et al., 2023), particularly for reconstructing oceanic anoxic events
94 (Connock et al., 2022; Rattanasriampaipong et al., 2024). However, the distributions,
95 environmental controls, and biological sources of sb/ob-GDGTs in terrestrial
96 environments remain poorly documented, hindering both mechanistic understanding of
97 their biosynthesis and their application as reliable proxies. This knowledge gap is
98 particularly critical in permafrost soils, where hydrological changes strongly regulate
99 redox conditions and carbon cycling.

100 The Qinghai-Tibetan Plateau (QTP), the world's largest high-altitude permafrost,
101 is highly sensitive to climate warming and characterized by substantial soil carbon
102 storage (Ding et al., 2017; Schädel et al., 2016). Driven by atmospheric precipitation
103 and seasonal snowmelt (Boike et al., 2013), elevated soil water content (SWC) can limit
104 oxygen diffusion, creating micro-oxic to anoxic conditions that restructure microbial
105 communities and biogeochemical processes (Marschner, 2021; Pett-Ridge & Firestone,
106 2005). Such shifts in microbial community composition are likely to perturb br-GDGT
107 distributions, complicating their application as paleoclimate proxies in water-saturated
108 soils (Liang et al., 2023; Raberg et al., 2024; Tang et al., 2024). Despite these
109 observations, the underlying biochemical mechanisms remain poorly understood,
110 particularly how bacterial membrane adaptations respond to redox variations in these
111 environments.

112 In this study, we investigate the distribution of sb/br/ob-GDGTs and their potential



113 microbial sources on the QTP by combining mass spectrometry-based lipid analysis
114 and high-throughput 16S rRNA gene sequencing. We aim to 1) characterize the
115 composition and distribution of these lipids in relation to environmental parameters, 2)
116 identify their potential microbial producers, and 3) evaluate the applicability of the
117 methylation index (i.e. $MI_{ob/br}$ and $MI_{ob/br/sb}$) in terrestrial environments.

118 **2. Materials and methods**

119

120 **2.1. Sample collection and physiochemical analyses**

121 Two soil cores were collected in July and August 2018 on the QTP, specifically at
122 Longren (LR, 30°30'N, 91°13'E, 4257 meters above sea level; hereafter m a.s.l.) and
123 NamCo (NVI, 30°43'N, 91°20'E, 4860 m a.s.l.) (Fig. 1a). Detailed site descriptions are
124 provided by Tang et al. (2023) and the results are shown in Table S1. The cores reached
125 depths of 2.0 m at LR and 1.5 m at NVI, with samples collected at 10 cm intervals. *In*
126 *situ* soil temperature was measured using a metallic thermometer. Visible plant roots
127 and organic debris were manually removed prior to processing. In total, 20 samples
128 were collected from LR and 13 from NVI. The soil samples were stored at –80°C until
129 further analysis.

130 Soil pH was measured following Ding et al. (2015). MilliQ water was added to the
131 samples in a 1:2.5 (g of soil: mL of water) ratio. The mixture was shaken to homogenize
132 the suspension and then allowed to settle. The pH of the supernatant was measured in
133 triplicate using a pH meter (Mettler Toledo) with a standard deviation < 0.05.

134 Soil water content (SWC) was determined gravimetrically (Evelt et al., 2009;
135 Tanriverdi et al., 2016). Defrosted samples were weighed to record wet weights, then
136 freeze-dried for 60 hours and reweighed to determine dry weights. SWC was calculated
137 using the following formula (Tanriverdi et al., 2016):

$$138 \quad \text{SWC (\%)} = (\text{wet weight} - \text{dry weight}) / \text{dry weight} \times 100$$

139



140 2.2. Lipid extraction and analysis

141 Total lipids were extracted from soil samples using a modified Bligh & Dyer
142 method (Sturt et al., 2004). Briefly, the freeze-dried samples were homogenized and
143 filtered through a 50-mesh sieve. Approximately 2 g of each sample was ultrasonically
144 extracted twice for 10 min each in a mixture of dichloromethane (DCM)/methanol
145 (MeOH)/phosphate buffer (pH 7.4; 2:1:0.8, v/v/v), followed by centrifugation at 1500
146 × g for 5 min to collect the supernatant. The remaining sample was then extracted twice
147 in another solvent mixture of DCM/MeOH/trichloroacetic acid buffer (pH 2; 2:1:0.8,
148 v/v/v). All supernatants were combined, and DCM and water were added to achieve a
149 final MeOH/DCM/H₂O ratio of 1:1:0.9. After incubation at 4°C overnight, the DCM
150 phase was collected as the total lipid extract (TLE) using a separatory funnel and dried
151 under a gentle N₂ stream.

152 An aliquot of each TLEs was fractionated into core lipids and polar lipids
153 following Oba et al. (2006) and Pitcher et al. (2009). Specifically, core lipids were
154 eluted from silica gel with 14 mL of a hexane/ethyl acetate mixture (1:1, v/v), and polar
155 lipids were subsequently eluted with 8 mL of MeOH. An internal standard of C₄₆-GTGT
156 (1.189 ng/μL, 50 μL) was added to each sample fraction. The fractions were dried under
157 a N₂ stream.

158 2.2.1. Total lipid analysis by UPLC-ESI-qTOF

159 Total lipids were analyzed using ultra-performance liquid chromatography
160 coupled with a quadrupole time-of-flight mass spectrometry with ion mobility,
161 equipped with an electrospray ionization source (UPLC-ESI-qTOF, SYNAPT G2-Si,
162 Waters) operated in positive mode. Compound separation was achieved on an Acquity
163 BEH C₁₈ column (1.7 μm, 2.1 × 150 mm) and the chromatographic conditions were
164 adapted from Wörmer et al. (2013). Eluent A was MeOH/H₂O/formic acid
165 (HCO₂H)/14.8 M aqueous ammonia (NH₃) at a ratio of 85:15:0.04:0.1 (v/v/v/v), and
166 eluent B was isopropyl alcohol (IPA)/MeOH/HCO₂H/14.8 M aqueous NH₃ at a ratio of
167 50:50:0.04:0.1 (v/v/v/v).

168 The total lipids were eluted using an initial isocratic hold at 100% A for 2 min,



169 followed by a 1-min linear gradient to 15% B. The gradient was then increased linearly
170 to 85% B from 3 to 27 min, and returned to 0% A at 27.1 min. The column was then
171 equilibrated with 0% A for 9.9 min, followed by a final equilibration with 100% A for
172 8 min, completing the total run at 45 min. The column temperature was maintained at
173 45°C and the flow rate was set at 0.3 mL/min. The MS settings were identical to those
174 described by Chen et al. (2022). Data were acquired in FAST-DDA (Data-Dependent
175 Acquisition) mode, with mass ranges of 100-2000 Da for MS¹ and 50-2000 Da for MS².
176 Lipids were identified based on retention time, accurate mass, and diagnostic ions in
177 MS² spectra (Liu et al., 2012) and the results are shown in Table S2. The peaks were
178 manually integrated for subsequent analysis, and representative MS² spectra are shown
179 in Fig. S1 and Fig. S2.

180 2.2.2. Core lipid analysis by HPLC-APCI-MS

181 The separated core lipids were analyzed using an Agilent 1200 series high-
182 performance liquid chromatography (HPLC), coupled to a 6135B quadrupole mass
183 spectrometry equipped with an atmospheric pressure chemical ionization (APCI)
184 source operating in positive mode. The chromatographic separation was carried out
185 using two BEH HILIC columns (2.1 × 150 mm, 1.7 µm, Waters) connected in tandem
186 (Hopmans et al., 2016), with eluent A (hexane) and eluent B (hexane: isopropanol, 9:1,
187 v/v). The elution gradient initially started with an isocratic hold of 82% A for 25 min,
188 then reduced to 65% A over the next 25 min. A linear gradient from 100% B to 82% A
189 was applied over 30 min, followed by a return to 82% A for 10 min, and re-equilibration
190 at this ratio for an additional 30 min. The flow rate was set at 0.2 mL/min, and the
191 column temperature was maintained at 30°C. The MS settings were identical to those
192 described by Schouten et al. (2007) and data were acquired in selected ion monitoring
193 mode at $m/z = 653.6, 743.6, 1022.0, 1020.0, 1018.0, 1036.0, 1034.0, 1032.0, 1050.0,$
194 $1048.0, 1046.0, 1302.3, 1300.3, 1298.3, 1296.3, 1294.3, 1292.3$. Peak integration was
195 performed using ChemStation software. Lipid concentrations were determined by
196 comparing peak areas with those of the internal standard C₄₆-GTGT (Huguet et al.,
197 2006), and results were reported as ng g⁻¹ dry weight soil (dws) (Table S3).



2.3. Proxy calculation

The proxies used in this study were as follows. The calculation was based on the relative abundance of the compounds:

Eq.	Proxy	Reference
1	$MI_{ob/br/sb} = (SB-980 + 2 \times SB-994 + 3 \times SB-1008 + 4 \times Ia + 5 \times IIa + 6 \times IIIa + 7 \times OB-1064 + 8 \times OB-1078 + 9 \times OB-1092 + 10 \times OB-1106 + 11 \times OB-1120 + 12 \times OB-1134) / (\sum sb-GDGTs + \sum br-GDGTs + \sum ob-GDGTs)$	(Liu et al., 2014)
2	$MI_{ob/br} = (4 \times Ia + 5 \times IIa + 6 \times IIIa + 7 \times OB-1064 + 8 \times OB-1078 + 9 \times OB-1092 + 10 \times OB-1106 + 11 \times OB-1120 + 12 \times OB-1134) / (\sum br-GDGTs + \sum ob-GDGTs)$	(Liu et al., 2014)
3	$MI_{ob} = (7 \times OB-1064 + 8 \times OB-1078 + 9 \times OB-1092 + 10 \times OB-1106 + 11 \times OB-1120 + 12 \times OB-1134) / (\sum ob-GDGTs)$	(Liu et al., 2014)
4	$MBT'_{5ME} = (Ia + Ib + Ic) / (Ia + Ib + Ic + IIa + IIb + IIc + IIIa)$	(De Jonge et al., 2014a)
5	$MBT'_{6ME} = (Ia' + Ib' + Ic') / (Ia' + Ib' + Ic' + IIa' + IIb' + IIc' + IIIa')$	(De Jonge et al., 2014a)

201

2.4. Bacterial community analysis, lipid–taxon correlation networks, and putative GDGT-related biosynthesis protein search

The detailed procedures for soil DNA extraction, sequencing and subsequent data processing were reported in Tang et al. (2023). In brief, total genomic DNA was extracted from approximately 0.2 g of homogenized fresh soil using the PowerSoil DNA Isolation Kit (MoBio Laboratories, USA). Bacteria and archaea 16S rRNA genes were amplified using the universal primer pair 515FmodF (5'-GTGYCAGCMGCCGCGGTAA-3') (Parada et al., 2016) and 806RmodR (5'-GGACTACNVGGGTWTCTAAT-3') (Apprill et al., 2015). Raw paired-end reads (2 × 300 bp) were generated using the Illumina MiSeq platform (Illumina, USA).

Raw sequences were processed by merging paired reads, quality filtering, primer trimming, and chimera removal using USEARCH 11.0.667 and VSEARCH 2.10.4



214 (Rognes et al., 2016). High-quality sequences were then denoised using the DADA2
215 plugin in the QIIME 2 pipeline (version 2020.2) with recommended parameters,
216 yielding single-nucleotide resolution based on error profiles within samples. High-
217 quality reads were taxonomically assigned using the Greengenes reference database
218 (McDonald et al., 2012). The bacterial sequences were then summarized into an
219 amplicon sequence variant (ASV) abundance table with taxonomic annotations for
220 downstream analyses. Only taxa with relative abundances $> 0.1\%$ and present in $> 25\%$
221 of samples were retained.

222 Spearman correlation analyses between the relative abundances of bacterial ASVs
223 or genera and sb/br/ob-GDGTs were performed in R software (version 4.0.2; R Project
224 for Statistical Computing; <http://www.r-project.org/>). Significant correlations were
225 identified using the “corr.test” function in the psych package (Revelle, 2024) with FDR-
226 adjusted p -value < 0.05 . ASV-level networks were constructed separately for positive
227 and negative correlations using thresholds of $|r| > 0.7$ and $p < 0.05$. In addition, a genus-
228 level correlation network was constructed using a threshold of $|r| > 0.6$ and $p < 0.05$.
229 Networks were visualized using Gephi (version 0.10.1, <https://gephi.org/>).

230 We selected genera with strong correlations ($|r| > 0.7$) with branched GDGTs.
231 Their NCBI Taxonomy ID (TaxID) were used to extract sequences and performed
232 functional annotation via BLASTp against the NCBI non-redundant database.
233 Specifically, BLASTp searches were performed to identify homologs of validated or
234 putative GDGT-related proteins (e-value $< 1e^{-50}$, identity $> 20\%$), including ElbD, Ger
235 and Mss, as well as archaeal isoprenoid GDGT biosynthetic enzymes, such as Tes and
236 Gmm (Garcia et al., 2024; Lloyd et al., 2022; Lorenzen et al., 2014; Sahonero-Canavesi
237 et al., 2022; Zeng et al., 2022). The (putative) functions of these proteins are listed in
238 Supplementary Table S5.

239

240 **2.5. Statistical analyses**

241 All statistical analyses were conducted in R software (version 4.5.3, [http://www.r-](http://www.r-project.org)
242 [project.org](http://www.r-project.org)). Figures were primarily generated using the ggplot2 package.



243 Environmental parameters between the NVI and LR sites were compared using the
244 Wilcoxon test, and principal component analysis (PCA) and redundancy analysis (RDA)
245 were performed using R packages.

246

247 **3. Results**

248

249 **3.1. Environmental parameters**

250 Distinct gradients in pH, *in situ* soil temperature and SWC were observed between
251 the two permafrost soil cores (NVI and LR; Fig. 1b). Samples from the NVI core were
252 alkaline, with pH ranging from 7.3 to 8.2 (7.8 ± 0.3 ; mean $\pm$ standard deviation, same
253 hereafter), while the LR core showed relatively lower, neutral-to-acidic pH values
254 ranging from 6.1 to 7.5 (6.5 ± 0.3). SWC was substantially lower in the NVI core (46
255 $\pm 9\%$) than in the LR core ($67 \pm 15\%$). The *in situ* soil temperatures of NVI ranged
256 from -0.9°C to 8.1°C ($1.5 \pm 2.9^{\circ}\text{C}$), while those of LR ranged from 4.9°C to 15.0°C
257 ($8.5 \pm 2.9^{\circ}\text{C}$).

258 The vertical profiles of temperature in both NVI and LR showed a declining trend
259 with depth (Fig. 2a and 2b). In the NVI core, SWC remained relatively low and stable,
260 with localized peaks exceeding 50% only in 80-110 cm (Fig. 2a). In contrast, SWC in
261 the LR core was generally higher, exceeding 50% in most samples and increasing
262 progressively with depth to a maximum near 150 cm before slightly decreasing in
263 deeper layers (Fig. 2b). These diverging thermal and hydrological regimes, coupled
264 with pH, provide a robust environmental gradient between NVI and LR cores.

265

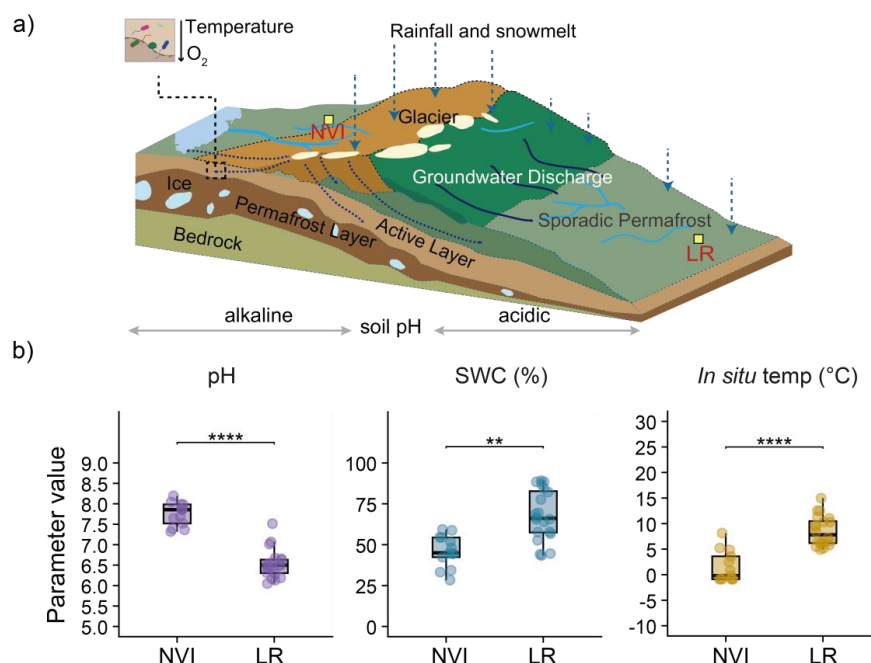


Fig. 1. Environmental and physiochemical characteristics of the study sites in the Tibetan Plateau. (a) Schematic representation of the geographic and geological features of the NVI and LR sampling sites (modified from Tang et al., 2023). **(b)** Comparison of soil pH, soil water content (SWC, %), and *in situ* temperature (°C) between NVI and LR permafrost soil cores. Statistical significance was assessed using Wilcoxon rank-sum tests (** $p < 0.01$, **** $p < 0.0001$).

3.2. Vertical variation of sb/br/ob-GDGTs in two permafrost soil profiles

Down-core variations in GDGT abundances and proxies of the NVI and LR core are shown in Fig. 2. In both soil profiles, br-GDGTs were predominant, followed by ob-GDGTs and sb-GDGTs. The average concentration of br-GDGTs in LR core was $40.6 \pm 26.8 \mu\text{g g}^{-1}$ dws, nearly 20-fold higher than that in NVI core ($2.1 \pm 0.9 \mu\text{g g}^{-1}$ dws) (Fig. 2). Similarly, ob-GDGTs and sb-GDGTs were more abundant in LR core ($5.39 \pm 5.34 \mu\text{g g}^{-1}$ dws and $1.10 \pm 1.14 \mu\text{g g}^{-1}$ dws, respectively) than in NVI core ($0.04 \pm 0.03 \mu\text{g g}^{-1}$ dws and $0.01 \pm 0.01 \mu\text{g g}^{-1}$ dws, respectively).

The two cores also differed in lipid composition. Non-cyclized br-GDGTs (predominantly IIa and IIIa) dominated in NVI core ($51 \pm 2\%$ of the total br-GDGTs),



283 whereas Ib and IIb were relatively more abundant in LR core ($46 \pm 11\%$) (Table S2).
284 Among ob-GDGTs, OB-1064 was the most abundant isomer in NVI core ($35 \pm 22\%$ of
285 the total ob-GDGTs), whereas OB-1106 predominated in the LR core ($28 \pm 15\%$). As
286 for sb-GDGTs, SB-966 was the major component in both cores, accounting for $60 \pm$
287 24% of the total sb-GDGTs in NVI and $61 \pm 13\%$ in LR.

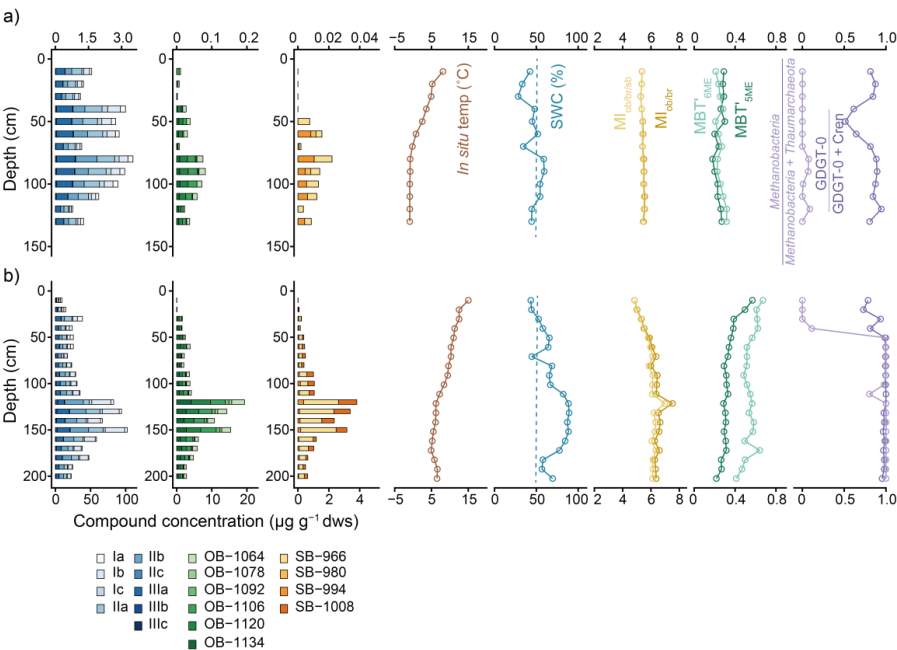
288 To further evaluate the environmental controls on lipid distributions, we compared
289 two sets of molecular proxies: MBT'_{5ME} and MBT'_{6ME} (De Jonge et al., 2014a), and
290 $MI_{ob/br}$ and $MI_{ob/br/sb}$ (Liu et al., 2014; Zeng et al., 2023). $MI_{ob/br}$ and $MI_{ob/br/sb}$ were
291 elevated in LR (6.17 ± 0.60 and 5.96 ± 0.48 , respectively) relative to NVI (5.44 ± 0.05
292 and 5.41 ± 0.04 , respectively). Both MBT'_{5ME} and MBT'_{6ME} indices were higher in LR
293 (0.33 ± 0.08 and 0.55 ± 0.06 , respectively) than in NVI (0.25 ± 0.04 and 0.26 ± 0.04 ,
294 respectively) (Fig. 2; Table S3).

295 Vertical profiles revealed distinct depth-dependent patterns. In the NVI core, lipid
296 concentrations remained low and stable, whereas in the LR core they increased
297 dramatically with depth and peaked at 120-150 cm, corresponding to the zone of highest
298 SWC. Furthermore, MBT' indices in both cores decreased slightly with depth,
299 consistent with the vertical *in situ* temperature gradient. In contrast, MI indices
300 exhibited a consistent upward trend, especially in the water-saturated deeper layers of
301 the LR core (Fig. 2).

302 To evaluate the redox states of the two profiles in the absence of *in situ* oxygen
303 measurements, we employed two proxies based on archaeal lipids and community
304 composition: the $GDGT-0/(GDGT-0 + Cren)$ ratio and the
305 $Methanobacteria/(Methanobacteria + Thaumarchaeota)$ ratio. GDGT-0 is a common
306 lipid in many archaeal taxa and is likely produced by diverse anaerobic archaeal
307 lineages in soils, such as methanogens and *Bathyarchaeota* in water-saturated soil
308 environments (Buckles et al., 2013; Schouten et al., 2013; Zheng et al., 2019).
309 Crenarchaeol (Cren) is a diagnostic biomarker for aerobic ammonia-oxidizing archaea
310 under the order *Nitrososphaeria* (formerly *Thaumarchaeota*) (Elling et al., 2025;



311 Leininger et al., 2006). The relative abundances of GDGT-0 and Cren may serve as
312 indicators for the proportion of anaerobic archaea and, by extension, oxygen availability
313 (Pei et al., 2021; Qin et al., 2015; Weber et al., 2018; Yang et al., 2019). On average,
314 the LR core exhibited stronger anaerobic characteristics than the NVI core (Fig. 2). The
315 GDGT-0/(GDGT-0 + Cren) ratio was consistently higher in the LR core, increasing
316 rapidly from 0.73 to 0.99 at a depth of 50 cm and remaining stable with increasing depth
317 (0.98 ± 0.01). This ratio remained low throughout the NVI core and increased slightly
318 at depths greater than 80 cm. Furthermore, we examined the microbial community ratio
319 of *Methanobacteria*/(*Methanobacteria* + *Thaumarchaeota*). *Methanobacteria* are
320 strictly anaerobic archaea and were abundant in deep sections of the LR core (Tang et
321 al., 2023). Therefore, this ratio may provide more direct evidence of soil redox
322 conditions. Consistent with the GDGT-based ratio, the
323 *Methanobacteria*/(*Methanobacteria* + *Thaumarchaeota*) ratio was substantially higher
324 in the LR core (0.79 ± 0.39) than that in the NVI core (0.02 ± 0.03).
325



326

327 Fig. 2. Vertical profiles of lipid concentrations, environmental parameters and relevant proxies with soil depth



328 at (a) NVI site and (b) LR site. Stacked bar charts show concentrations ($\mu\text{g g}^{-1}$ dws)) of br-(blue), ob-(green),
329 and sb-(orange) GDGTs. Line plots illustrate depth-dependent variations in *in situ* temperature ($^{\circ}\text{C}$), SWC
330 (%), proxies and ratios derived from lipid and sequencing data, including $\text{MI}_{\text{ob/br}}$ and $\text{MI}_{\text{ob/br/sb}}$, MBT'_{SME} and
331 MBT'_{6ME} , $\text{GDGT-0}/(\text{GDGT-0} + \text{Cren})$ and *Methanobacteria*/(*Methanobacteria* + *Thaumarchaeota*).

332

333 3.3. Correlations between sb/br/ob-GDGTs and bacterial communities

334 The co-occurrence network analysis revealed strong relationships between the
335 relative abundances of the bacterial communities and sb/br/ob-GDGTs (Fig. 3), with
336 similar prevalent in positive and negative ones (Fig. 3a, 3b). These associations also
337 differed among bacterial groups at the genus level: anaerobic bacteria (13.51% of the
338 total nodes) were mainly positively correlated with lipids, whereas aerobic bacteria
339 (33.78%) were predominantly negatively correlated (Fig. 3c). The bacterial taxa
340 associated with distinct lipid classes showed remarkable convergence, indicating a
341 shared microbial signature (Fig. 3d). Eight genera, including anaerobes such as
342 *Syntrophus*, *LCP-26*, *LCP-6*, *Treponema*, *SJA-88*, and *C1_B004*, exhibited positive
343 correlations with sb/br/ob-GDGTs. In contrast, a larger group of 36 bacterial genera
344 showed consistent negative correlations across all lipid classes.

345 BLASTp alignment revealed that several bacterial genera harbor genetic potential
346 for GDGT biosynthesis (Fig. 3 and Table S5). Among the identified GDGT-related
347 homologs, glycerol ester reductase (Ger) is involved in the conversion of an ester bond
348 to an ether bond (Sahonero-Canavesi et al., 2022), whereas membrane-spanning lipid
349 synthase (Mss) is responsible for the coupling of the alkyl chains to form bacterial
350 membrane-spanning lipids (Sahonero-Canavesi et al., 2026; Sahonero-Canavesi et al.,
351 2022). Notably, homologs of both Ger and Mss were detected in the anaerobic genera
352 *LCP-26*, *Syntrophus*, and *Treponema* (Table S5), suggesting that these taxa may have
353 the capacity to synthesize br-GDGTs in the anaerobic soils investigated here. In addition,
354 these genera also showed positive correlations with sb/br/ob-GDGTs, further
355 supporting their potential as biological sources of sb/br/ob-GDGTs.

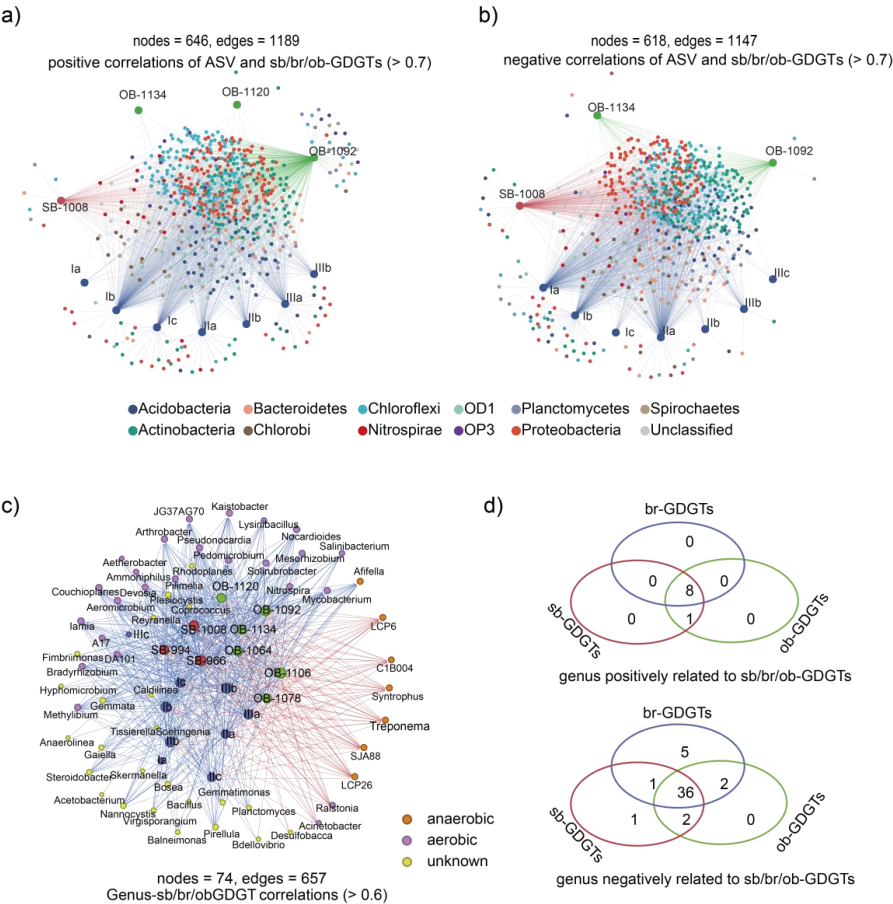


Fig. 3. Co-occurrence network analysis based on correlations between relative abundances of bacterial ASV and sb/br/ob-GDGTs, showing positive (a) or negative (b) correlations (filtered by $|r| > 0.7$, $p < 0.05$). In these panels, edge colors distinguish the GDGT types: blue for br-, green for ob-, and red for sb-GDGTs. (c) The co-occurrence network depicts correlations between bacterial groups at the genus level and sb/br/ob-GDGTs ($|r| > 0.6$, $p < 0.05$). Node colors indicate the oxygen requirements of bacteria, while red and blue edges represent positive and negative correlations between microbial communities and sb/br/ob-GDGTs, respectively. (d) Venn diagrams illustrate the overlap of bacterial taxa associated with sb/br/ob-GDGTs based on the correlations shown in (c).

4. Discussion



4.1. Soil water content drives branched GDGT distributions via microbial community succession

Although branched GDGTs are widely distributed in high-altitude soils (Huguet et al., 2017; Liang et al., 2023; Tang et al., 2024; Yang et al., 2026), the environmental factors controlling their distributions remain poorly constrained. Previous studies have shown that a range of environmental variables, including temperature, soil pH, vegetation, and soil physiochemical properties, significantly influence the distribution and abundance of brGDGTs (Ding et al., 2015; Halffman et al., 2022; Liang et al., 2023; Pei et al., 2021; Raberg et al., 2024; Yang et al., 2026). Among these factors, SWC is a key physiochemical parameter that regulates other environmental variables, such as nutrient availability and redox conditions, thereby shaping microbial community composition in soil environments. The influence of soil moisture on brGDGT distributions has been observed in soils from arid to subhumid regions (Dang et al., 2016; Loomis et al., 2011; Sun et al., 2016; Wang et al., 2014), as well as in water-saturated environments such as peat bogs (Dirghangi et al., 2013; Raberg et al., 2024; Rao et al., 2022; Weijers et al., 2009) and permafrost soils (Tang et al., 2024). In semi-arid and arid soils, the effects of SWC on brGDGT distributions are primarily associated with changes in the CBT index and the degree of methylation of 6-methylated brGDGTs (Dang et al., 2016; Yang et al., 2026). In wet soils, SWC has been shown to influence the methylation indices (MBT'/MBT'_{SME}), potentially by altering soil heat capacity, which can decouple soil temperature from air temperature (Dang et al., 2016; Naafs et al., 2017) or the bacterial community that produces br-GDGTs (Tang et al., 2024).

In this study, our results demonstrated that SWC was the primary environmental gradient controlling the composition and methylation degree of sb/br/ob-GDGTs in the Tibetan Plateau peatlands (Fig. S3). Principal component analysis revealed that the major variance in lipid distributions was closely associated with SWC, with highly methylated br-GDGTs (e.g., Ib, IIb, IIIb) and all ob-GDGTs being significantly enriched in high-SWC samples (Fig. S4). Furthermore, both the $MI_{ob/br}$ ($R^2 = 0.65$, $p < 0.001$) and $MI_{ob/br/sb}$ ($R^2 = 0.63$, $p < 0.001$) indices showed strong positive correlations



397 with SWC (Fig. 4), confirming a systematic shift of the GDGT assemblage toward a
398 higher degree of methylation as soil moisture increases.

399 One plausible mechanism underlying the relationship between SWC and sb-, br-,
400 and ob-GDGT distributions is that increased SWC drives shifts in soil redox conditions
401 via physical processes. In wet to water-saturated soils, an increased proportion of water-
402 filled pore space impedes the diffusion of gaseous oxygen (Harlan, 1973), thereby
403 shifting the soil environment from predominantly aerobic to microoxic or even anoxic
404 conditions (Linn & Doran, 1984; Ponnampertuma, 1972; Vepraskas & Faulkner, 2001).
405 In permafrost soils, particularly in thermokarst wetlands, seasonal thawing of the active
406 layer often leads to soil saturation or waterlogging. Under these conditions, oxygen
407 depletion can occur rapidly, leading to reducing conditions in many thawed permafrost
408 environments. Although culture experiments suggested that *Acidobacteria* grown under
409 aerobic conditions are biological sources of brGDGTs (Chen et al., 2022; Halamka et
410 al., 2023), oxygen limitation may stimulate the production of branched GDGTs in
411 certain strains (Halamka et al., 2021). This observation implies that oxygen depletion
412 may facilitate the anaerobic production of brGDGTs, consistent with the increased
413 abundance of brGDGTs observed in diverse anaerobic environments. Moreover, we
414 observed high abundances of ob-GDGTs in soils with high SWC, consistent with
415 previous observations of elevated ob-GDGT abundance and enhanced methylation in
416 oxygen minimum zones and marine sediments (Liu et al., 2014; Xie et al., 2014; Zeng
417 et al., 2023).

418 This moisture-mediated redox shift acts as a primary driver of microbial
419 community structure and metabolism in soils, potentially resulting in a transition of
420 brGDGT-producing bacteria from predominantly aerobic taxa in surface soils to
421 anaerobic taxa in deeper or water-saturated soils. This interpretation is consistent with
422 previous studies on the potential biological sources of brGDGTs (Liang et al., 2023;
423 Tang et al., 2023). This mechanism can also be supported by co-occurrence network
424 results, which show that branched GDGTs are positively correlated with specific
425 anaerobic bacterial communities, such as *LCP-26*, *Syntrophus* and *Treponema* (Fig. 3c;



Section 3.3). Furthermore, functional gene prediction provides additional support for this notion, as homologs of enzymes potentially related to GDGT biosynthesis were aligned in these positively correlated anaerobic genera (Table S5; Section 3.3), indicating that these taxa could have the potential to produce br-GDGTs in anaerobic soils in this study (Sahonero-Canavesi et al., 2026).

Our findings suggest that elevated SWC may promote micro-oxic conditions by limiting oxygen diffusion, which in turn could drive the enrichment and succession of obligate and facultative anaerobic bacterial communities with the potential to produce more highly methylated branched GDGTs in Tibetan Plateau permafrost soils.

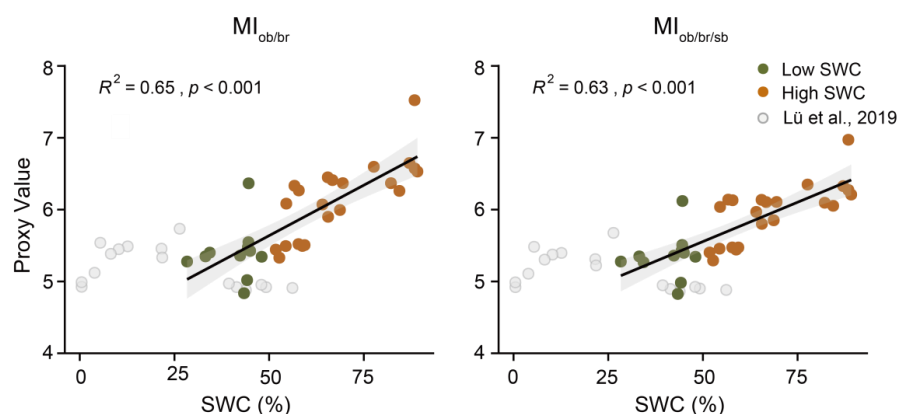


Fig. 4. Linear regressions between SWC (%) and $MI_{ob/br}$ and $MI_{ob/br/sb}$ proxies. Samples are categorized into low SWC (<50%, green) and high SWC (>50%, orange). Shaded areas denote 95% confidence intervals. Both proxies show significant correlations with SWC ($R^2 \geq 0.6$, $p < 0.001$). Grey circles represent reference data from Lü et al. (2019).

4.2. Different patterns of branched GDGTs between marine and terrestrial environments

By compiling published datasets, we observed systematic differences in branched GDGT distributions between terrestrial and marine environment (Table S4). In terrestrial environments, branched GDGTs are overwhelmingly dominated by br-GDGTs, whereas marine environments are characterized by high abundances of sb- and ob-GDGTs (Fig. 5a) (Liu et al., 2014; Lü et al., 2019; Zeng et al., 2023). Consequently,



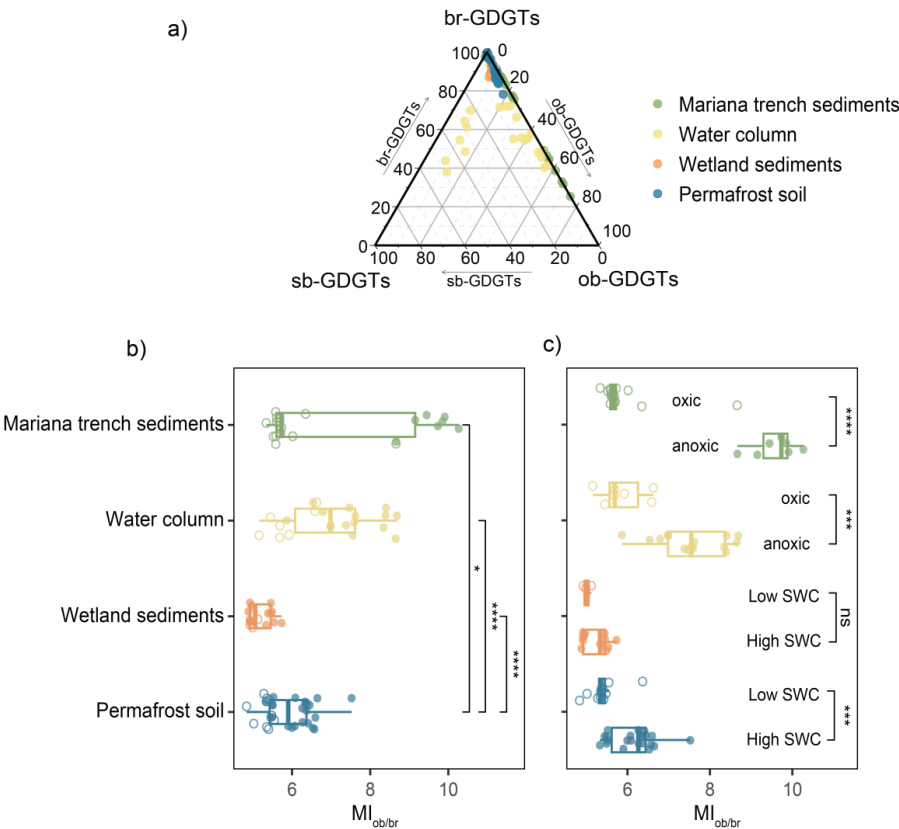
448 marine environments exhibit markedly higher degrees of methylation, as reflected by
449 elevated $MI_{ob/br}$ values, compared to terrestrial environments (Fig. 5b). Notably, the
450 $MI_{ob/br}$ index is significantly higher in anoxic environments than in oxic environments
451 (Fig. 5c).

452 This divergence may be driven by two mechanisms: shifts in the microbial sources
453 of branched GDGTs and distinct environmental adaptations. Although terrestrial and
454 marine environments share several major bacterial phyla that are related with branched
455 GDGTs, including *Chloroflexi* and *Proteobacteria*, the dominant genera associated with
456 branched GDGTs differ substantially between these environments. For example,
457 *SAR202*, *Rhodospirillales* and *DG-56* are strongly correlated with branched GDGT
458 abundance in marine sediments (Zeng et al., 2023), whereas *LCP-26*, *Syntrophus*, *LCP-*
459 *6*, *Treponema*, and *CI_B004* are associated with branched GDGTs in peat soils.
460 Alternatively, such divergence could also be attributed to differences in biosynthetic
461 capacity for methylations among source microorganisms in diverse environments,
462 which may be mainly controlled by the gene responsible for brGDGT methylation. A
463 recent study has identified a gene encoding glycerol monoalkyl glycerol tetraether
464 (GMGT) methylase (Gmm) in archaea, and homologs of this enzyme are also present
465 in multiple bacterial phyla (Garcia et al., 2024). Although the role of these homologs in
466 Bacteria domain has not yet been experimentally verified, the structural similarities
467 between membrane-spanning lipids in archaea and bacteria indicate that bacterial Gmm
468 homologs could probably also involve in the production of methylated brGDGTs.
469 Based on the distinct patterns of branched GDGTs, we infer that marine-sourced
470 bacteria may possess a greater capacity for producing highly methylated brGDGTs
471 compared to terrestrial sources due to the diversification of the genes involving in the
472 formation of methylation, probably *gmm* gene homologs. However, more work is
473 needed to ascertain this hypothesis including identification of the gene responsible for
474 brGDGT methylation biosynthesis and phylogenetic analysis across diverse taxa and
475 environments. On the other hand, lipid distributions likely reflect microbial adaptations
476 to specific environmental conditions (Ding et al., 2022; Safronova et al., 2024; Tang et



477 al., 2024). In deep marine environments, branched GDGT-producing bacteria may
478 synthesize more highly methylated GDGTs in response to low temperatures, high
479 hydrostatic pressure, and oligotrophic (nutrient-poor) conditions (Xiao et al., 2020;
480 Xiao et al., 2024). The increase in the degree of methylation of branched GDGTs with
481 water depth is likely a physiological strategy for maintaining membrane stability and
482 functionality under the high-pressure and low-temperature conditions of the deep sea
483 (Tamby et al., 2026; Tamby et al., 2023). Terrestrial brGDGT distributions are
484 influenced not only by pH and ambient temperature, but also by SWC-driven variations
485 in oxygen availability (Liang et al., 2026; Tang et al., 2024).

486 Consequently, the differential patterns in sb/br/ob-GDGTs in terrestrial and marine
487 environments likely reflect variations in habitat-specific microbial communities. At the
488 same time, these distributions are likely shaped by multiple environmental factors,
489 including temperature, pH, hydrostatic pressure, and oxygen availability.



490

491 **Fig. 5. Comparative analysis of sb/br/ob-GDGT distributions and $MI_{ob/br}$ across diverse environments. (a)**
492 **Ternary plot showing the relative abundances of GDGTs in marine and terrestrial settings. (b) Variations in**
493 **$MI_{ob/br}$ across different habitats. (c) Comparison of $MI_{ob/br}$ within specific niches under varying oxygen (oxic**
494 **vs. anoxic) or moisture (low vs. high SWC) conditions. Data sources: Mariana Trench sediments (Zeng et al.,**
495 **2023); water column (Liu et al., 2014); wetland sediments (Lü et al., 2019); and permafrost soil (this study).**
496 **Statistical significance: * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$; ns: no significance.**

497

498 **4.3. Evaluate the $MI_{ob/br}$ as an indicator for soil water content-mediated redox** 499 **changes**

500 Soil water content (SWC) is one of the most important factors controlling physical,
501 chemical, and biological processes in soils. In permafrost regions, SWC can change
502 dramatically due to seasonal thaw and hydrological reorganization (Liljedahl et al.,



2016). Variations in SWC can substantially alter oxygen diffusion and availability, thereby mediating redox stratification in permafrost soils. These changes, in turn, regulate microbial carbon turnover and the balance between CO₂ and CH₄ production (Carlsen et al., 2025; Dang et al., 2026; Olefeldt et al., 2021; Turetsky et al., 2020). Therefore, investigating SWC-driven redox changes is critical for understanding biogeochemical processes and their implications for climate change in permafrost regions. To date, several inorganic and organic proxies have been proposed to track soil redox dynamics. For example, changes in redox potential and redox-sensitive solutes such as NO₂⁻, Mn²⁺, Fe²⁺, and SO₄²⁻ have been widely used to characterize soil reduction processes, while organic moieties such as phenols and quinones in soil organic matter can also record redox transformations (LaCroix et al., 2021; Mansfeldt, 2004). Increasing evidence suggests that branched GDGT distributions are also influenced by oxygen availability (Dang et al., 2016; Tang et al., 2024). For instance, IR_{6ME}, MBT'_{5ME}, MBT'_{6ME}, and CBT indices have been shown to correlate with oxygen availability, with lower oxygen levels generally associated with increased cyclization and reduced methylation of brGDGTs (Pei et al., 2021; Weber et al., 2018). In this study, we observed that MBT'_{5ME} and MBT'_{6ME} were positively correlated with *in situ* soil temperature (Fig. 2), whereas MBT'_{5ME} also showed significant relationships with SWC ($p < 0.01$; Fig. S5). Currently, there is no direct culture evidence that bacteria adapt to oxygen-limited conditions by increasing lipid methylation. Therefore, we propose that the observed response of methylation more likely reflects shifts in microbial community composition rather than physiological adaptation at the cellular level. These results suggest that soil waterlogging exerts strong controls on redox potential (Eh), pH dynamics, electron-acceptor availability, and substrate regimes within soil microsites (Husson, 2013; Marschner, 2021; Merino et al., 2021; Ponnamperna, 1972), thereby reshaping branched GDGT-producing microbial communities toward the taxa that preferentially synthesize more highly methylated branched GDGTs under anaerobic conditions.



531 More importantly, novel branched GDGT-based proxies, including methylation
532 indices derived from br- and ob-GDGTs (e.g., MI, MOB, MI_{ob} and MI_{ob/br}), have
533 recently been developed to indicate redox conditions in marine environments (Connock
534 et al., 2022; Liu et al., 2014; Rattanasriampaipong et al., 2024; Xiao et al., 2024).
535 Consistent with observations from marine environments, MI_{ob/br} and MI_{ob/br/sb} in the
536 permafrost soil profiles increased with increasing SWC and were significantly
537 positively correlated with SWC ($p < 0.001$), particularly when SWC exceeded 50%
538 (Fig. 4). This pattern suggests that these indices may also serve as indicators of redox
539 conditions in soils. Importantly, the downcore trends of MI_{ob/br} and MI_{ob/br/sb} closely
540 paralleled those of archaeal indicators, including the GDGT-0/(GDGT-0 + Cren) and
541 *Methanobacteria*/(*Methanobacteria* + *Thaumarchaeota*) ratios (Fig. 2). Collectively,
542 our findings highlight MI_{ob/br} and MI_{ob/br/sb} as independent proxies for tracking SWC-
543 mediated redox changes in dynamic permafrost ecosystems, with potential applications
544 in other anaerobic depositional environments, such as peatlands.

545

546 **5. Summary and Conclusions**

547 Based on a comprehensive analysis of sb/br/ob-GDGTs in two permafrost peatland
548 soil profiles from the Tibetan Plateau, this study reveals that soil moisture is the primary
549 environmental control on the composition and methylation degree of these bacterial
550 lipids. Elevated SWC drives redox changes by limiting oxygen diffusion, which in turn
551 could stimulate the production of sb/br/ob-GDGTs, resulting in significantly higher
552 values of the methylation indices, MI_{ob/br} and MI_{ob/br/sb}. This finding extends the
553 application of ob/sb-GDGTs as redox-sensitive proxies from marine systems to
554 terrestrial permafrost environments.

555 Co-occurrence network analysis further demonstrates that the distributions of
556 these lipids are strongly associated with specific anaerobic bacterial communities,
557 including genera such as *Syntrophus*, *LCP-26*, and *Treponema*. Although the production
558 of sb/br/ob-GDGTs in marine and terrestrial ecosystems appears to be primarily
559 governed by low-oxygen conditions, the specific microbial taxa differ from those in



560 marine settings.

561 Consequently, the $MI_{ob/br}$ index is suggested as a promising proxy for
562 reconstructing soil redox dynamics driven by moisture variation. This tool may aid in
563 assessing past hydrological changes in warming permafrost regions, thereby improving
564 our understanding of permafrost carbon-cycle feedback to climate change.

565

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574

575 **Conflict of interest**

576 The authors declare no competing financial interests.

577



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