Branched glycerol dialkyl glycerol tetraethers and paleoenvironmental reconstruction in Zoigê peat sediments during the last 150 years

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Branched glycerol dialkyl glycerol tetraethers (GDGTs) were analyzed in peat samples taken from the Zoigê grassland, located on the eastern margin of the Qinghai-Tibetan Plateau. The mean annual air temperature (MAAT) and pH in the 210Pb-dating peat core were reconstructed based on the cyclization of branched tetraethers (CBT) and methylation of branched tetraethers (MBT) indices for the last 150 years. The results showed that the MAAT and pH values involved three stages of variation over the last 150 years. The pH was stable and the MAAT decreased slowly by about 1°C from 1851 to 1927. The pH decreased rapidly by 0.6 and the MAAT decreased significantly by 2.5°C between 1927 and 1979. The pH and MAAT increased by 0.8 and 5°C, respectively, since the 1980s. The reconstructed temperatures correspond well to the mean winter temperatures reconstructed using tree-rings from the Jiuzhaigou Valley, situated at the boundary between the Qinghai-Tibetan Plateau and the Sichuan Basin. The estimated MAAT was similar to the temperatures reconstructed using tree-rings, indicating an increasing trend, as shown by local instrumental records from 1957 to 2001. These results provide further confidence in the application of proxies based on glycerol dialkyl glycerol tetraethers in peat records for continental paleoclimate reconstruction.

Reconstructions of paleoclimates and paleoenvironments can help us to better understand the variation processes and mechanisms of the Earth’s systems and effectively reduce the uncertainties in these predictions by providing a basis for climatic and environmental predictions. Ice-core, loess, tree-ring, coral, and marine and lacustrine sedimentary records have been used for paleoclimate reconstruction. The key to establishing high-resolution climatic and environmental sequences ingeological history is to search for sensitive climatic and environmental proxies. Peat core sediments are also an important geological record. Peat core sediments are mainly accumulated in situ by plant remains and the burying process is relatively simple. The organic-matter content of these plant remains is relatively abundant, thus the remains are a good indicator to study the paleoenvironment/paleoclimate in terms of molecular organic geochemistry data.

Lipids (n-alkane, fatty alcohol, fatty acid, sterone, triterpenoid and linear-chain ester) in peat core sediments, isotopes of C, H, and O in celluloses, peat core humification rates and sporopollen fossils are good biomarkers for paleoenvironmental reconstructions [1–8]. Although these climatic proxies can be used to reconstruct paleoenvironments, providing information about changes in the paleoenvironment/paleoclimate (dry or wet, cold or warm), they cannot be used to calculate temperature values quantitatively.

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Moreover, most proxies show an indeterminate relationship with environmental factors (temperature, precipitation and CO₂ partial pressure); hence future work is needed to perfect these.

In recent years, the methylation of branched tetraethers (MBT) index and the cyclization of branched tetraethers (CBT) ratio, based on the distribution of glycerol dialkyl glycerol tetraethers (GDGTs), have been developed [9–12]. At present, two classes of GDGTs, namely isoprenoid GDGTs (GDGT-0–GDGT-4 and crenarchaeol) and branched GDGTs (I–III) (their molecular structures are shown in Figure 1), are being extensively studied. Isoprenoid GDGTs are biosynthesized exclusively by Archaea and are one of the biomarkers of Archaea [13]. The exact origin of the branched GDGTs is ambiguous, but it is likely that branched GDGTs are produced by bacteria in soil and peat [14]. Weijers et al. [9] described the relationship between the relative distributions of branched GDGTs and local environmental factors (temperature and pH) using 134 globally distributed soil samples from 90 different locations and showed that mean annual air temperature (MAAT) and soil pH were the major factors affecting the distribution of GDGTs. The amount of cyclopentane in the branched GDGTs is related to soil pH, whereas that of methyl groups is related to both MAAT and soil pH. Based on these observations, two indices were proposed for quantifying the distributions of branched GDGTs: the CBT index, reflecting soil pH, and the MBT index for both MAAT and soil pH. The indices are defined in formulas 1–4.

Peterse et al. [12] determined these indices for soils sampled from a transect in the peripheries of two hot springs in California. The CBT values of these geothermally heated soils showed good correlation with pH \((R^2 = 0.76)\), also the relationship between MBT, soil pH and temperature for the geothermally heated soils was similar to that of the global data set [9]. The results confirmed the dependence of the MBT index on soil temperature and pH and supported the applicability of the MBT/CBT indices as proxies for continental paleotemperatures and soil pH values in the past. Branched GDGTs have been used to reconstruct continental temperatures and soil pH values for several areas and geological times, for example, the last glacial-interglacial transition period in the Congo Basin, Africa [15], the Paleocene-Eocene thermal maximum from Arctic Ocean sediments [16], and at the Oligocene-Eocene boundary in Greenland [11]. Furthermore, Peterse et al. [17] applied these proxies to paleoclimate reconstruction in high-latitude environments with a MAAT of < 0°C. Although branched GDGT concentrations in the soil are relatively low, reconstructed MAATs based on the MBT/CBT proxies are ca. -4°C, close to the measured MAAT (ca. -6°C).

In this study, we measured the GDGT contents in Zoigê peat samples, calculated the MBT and CBT proxies by applying them to a peat core, and compared the estimates for the last 150 years with regional instrumental records of air temperatures and pH. The results provided some heuristic data for reconstruction of the continental paleoenvironment. The precisions of CBT and MBT computation are 0.014 and 0.008, respectively, and those of the pH and MAAT values obtained by conversion are ±0.04 and ±0.3°C, respectively.

**Figure 1**  Molecular structures of isoprenoid and branched GDGTs.
1 Materials and methods

1.1 Sampling and dating

The Hongyuan peat is located on the eastern edge of the Qinghai-Tibetan Plateau, in the northeast of Sichuan, in the middle of the Ngawa Tibetan and Qiang Autonomous Prefecture; it is situated in China’s biggest moss-land plateau – the Zoigê peat area. It is a subalpine meadow prairie belt consisting of herbaceous peat [18,19]. In the Hongyuan area, the prevailing climate is a monsoon climate characteristic of a continental plateau in a frigid-temperate zone, with short spring and autumn periods, long winters, and no summer. In this area, the annual mean temperature is 0.6–1.2°C and the annual mean precipitation is 560–860 mm. The climate is further characterized by a great difference in temperature between day and night, sufficient rainfall, and contemporaneous rainy and hot seasons [19,20].

The sampling site (32° 46′N, 102° 30′E) is located 2 km west of Hongyuan County in the Zoigê peat-core area in Hongyuan, Sichuan, at an altitude of 3510 m above sea level. The sampling tool was a stainless steel Wardenaar. The sample column was 25 cm long. The whole sample column was put into a PVC tube and kept at −10°C in the laboratory. The sample was divided longitudinally into two parts: one was used for 210Pb dating, and the other (subsampled at intervals of 0.5 cm) was used for GDGTs testing.

The age model was established using 210Pb data from the Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences (Figure 2), and the sample core was dated in the constant initial concentration mode [21–24].

![Figure 2](image)

**Figure 2** 210Pb profile (uns: unsupported. The amount of 210Pb, in a sediment sample can be calculated by measuring both 210Pb and 226Ra).

The precipitation rate is 0.16 cm/a, and the sample core time span is 1851–2006.

1.2 Methods

(i) Pretreatment method. Samples for lipid analysis were freeze dried and powdered with a mortar and pestle prior to extraction. Approximately 500 mg of freeze-dried sample, with a moderate amount of C46-GDGT (standard material, see the structure in Figure 1), were extracted by Soxhlet extraction with dichloromethane (DCM)/methanol (MeOH) (2/1, v/v) for 72 h. The solvent was evaporated and the extract was separated by Al2O3 column chromatography with hexane/DCM (9/1, v/v) and DCM/MeOH (1/1, v/v) as sequential eluents. The polar fraction (DCM/MeOH) was concentrated by rotary evaporation, redissolved in hexane/isopropanol (99/1, v/v), and filtered using a PTFE 0.45 μm filter. The filtrate was tested by high-performance liquid chromatography/atmospheric pressure chemical ionization −mass spectrometry (HPLC/APCI−MS).

(ii) Instrumental methods. The HPLC/MS method used was modified from that described by Hopmans et al. [25], as discussed by Schouten et al. [26].

(1) Chromatography conditions. Analyses were performed using an Agilent 1200 series liquid chromatograph equipped with an auto-injector and ChemStation chromatography management software. Separation was effected with a Prevail Cyano column (2.1 mm × 150 mm, 3 μm; Alltech, Deerfield, USA) kept at 30°C. The injection volume was 3 μL. GDGTs were eluted isocratically with 99% hexane and 1% propanol for 5 min, followed by a linear gradient to 2% propanol over 40 min. The flow rate was 0.2 mL/min. After each analysis, the column was cleaned by back-flushing hexane/propanol (90:10, v/v) at 0.2 mL/min for 15 min.

(2) Mass spectrometry conditions. Detection was achieved using atmospheric pressure, positive ion chemical ionization–Agilent 6410 triple quad mass spectrometer (APCI−MS). The conditions were: nebulizer pressure 60 psi; vaporizer temperature 300°C; drying gas (N2), flow rate 5 L/min, temperature 200°C; capillary voltage 2500 V; and corona 5 μA. GDGTs were detected via single-ion monitoring (SIM) of their [M + H]+ and [M + H]+ + 1 [i.e. protonated molecular ions (m/z values are shown in Figure 2) and first isotope peak ions] and quantified by integration of the peak areas [26].

Absolute concentrations were calculated using a newly synthesized internal standard material (C46-GDGT) (refer to the method described by Huguet [27]). The relative response factors (RRF) of each GDGT and the internal standard material were not measured, so only semi-quantitative results were achieved.

GDGT indices were calculated as follows [9], where the roman numerals correspond to the GDGT structures shown in Figure 1:
\[ \text{MBT} = \frac{([I_a] + [I_b] + [I_c])}{([I_a] + [I_b] + [I_c]) + ([II_a] + [II_b] + [II_c])} \times ([II_a] + [II_b] + [II_c]) \times \text{MAAT} + 0.122 + 0.187 \times \text{CBT} + 0.020 \times \text{MAAT} \]

2 Results and discussion

2.1 GDGTs composition and structural characteristics in the profile

Five isoprenoid and nine branched GDGTs were detected in the samples. The abundances of isoprenoid GDGTs are relatively low (0.001–0.63 μg/g, Figure 3(a)), accounting for about 3% of the total GDGTs, including GDGT-0, GDGT-1, GDGT-2, GDGT-3, and crenarchaeol. GDGT-0 is the most abundant isoprenoid GDGT, accounting for 62%–100% of the total isoprenoid GDGTs. Isoprenoid GDGTs possessing more than four cyclopentane groups were not examined. The above results are similar to those in the published literatures for soil and peat samples [9,28,29].

The concentrations of branched GDGTs vary between 1.8 and 20.3 μg/g, accounting for 96%–100% of the total GDGTs (Figure 3(b)). The examined branched GDGTs are mainly GDTG-I, GDTG-II, and GDTG-III (Table 1), with the highest concentration being that of GDTG-II and the lowest concentration that of GDTG-III. The concentrations tend to decrease as the number of cyclopentane groups in the GDGTs changes from 0 to 2. Branched GDGTs were discovered in a Dutch Holocene peat deposit and identified by Sinninghe Damsté et al. [30]. Weijers et al. [14] found that the stereoconfigurations of the glycerol moieties of the membrane lipids were identical to those of the glycerol moieties synthesized by bacteria. Thus, it is likely that the branched GDGTs are produced by bacteria. Branched GDGTs occur ubiquitously in soils [31,32], peats [31], lakes, and coastal marine sediments [31,33] throughout the world. It was initially thought that all of the branched GDGTs in lakes and coastal marine sediments were deposited after being transported by rivers from land [34,35]. Recent studies suggest that at least part of the branched GDGTs in marine sediments may be produced in situ [17,36–38].

2.2 pH and MAAT

In recent years, branched GDGTs in marine sediments have been used to reconstruct continental paleotemperatures and soil pH values in the last glacial-interglacial transition period in the Congo Basin, Africa [16], in the Paleocene-Eocene thermal maximum from Arctic Ocean sediments [15], and at the Oligocene-Eocene boundary in Greenland [11]. Branched GDGTs in lake sediments have also been used to reconstruct paleoenvironments. For example, Zink et al. [39] found that the MBT index was a good tool for reconstructing continental paleotemperatures by comparing the distributions of branched GDGTs in lake sediments.
deposits and recorded temperatures in New Zealand. Tyler et al. [36] reconstructed pH and MAAT values for approximately the past 400 years using a recent sedimentary GDGT profile from Lochnagar lacustrine deposits. The MBT/CBT proxies have been applied successfully to both marine and lacustrine deposits, but, to our knowledge, they have not been applied to the dating of peat-core deposits. In this research, we discussed the usefulness and potential application of these proxies in peat-core deposits.

In this research, the calculated CBT and MBT indices are 0.583–0.865 and 0.260–0.337, respectively (Figure 3(c) and (d)); Table 1). According to formulas (2) and (3), the pH values in the Zoigê peat core for the last 150 years are within the range 6.4–7.2 (Figure 3(e)). The pH values of the remaining soils were measured according to standard methods, i.e. in a peat/water mixture (1/2.5, m/v) [17]. The pH obtained by this method for the surface peat is 6.9, i.e., slightly lower than the calculated result obtained using the CBT index (7.1). The values deduced from the CBT index suggest that the pH variations could involve three periods in the last 150 years: in the period 1851–1927, the pH was stable at 7.0, reflecting the situation in this area — an uninhabited peat area before 1930 [40]; in the period 1927–1979, the pH decreased from 7.0 to 6.4, possibly because of deforestation in northwestern Sichuan Province, deforestation for farmland reclamation and the development of farmland since the 1920s [41], leading to extensive deposition of humus (mainly humic acid and fulvic acid); from 1979 to the present, the pH has increased by a large margin from 6.4 to 7.2. The steady increase in pH in the last 30 years could possibly be attributed to disturbance by human activities, resulting in decreased amounts of humic acid [42,43].

According to formula (4), the MAAT changed from −0.4°C to 4.7°C (Figure 4(d)). The MAAT variation, as defined by the CBT/MBT indices, could also involve three periods in the last 150 years: in the period 1851–1927, the MAAT varied over a small range from 2.8°C to 3.8°C (decreasing slowly by approximately 1°C); in the period 1927–1979, the MAAT varied between −1.2°C and 2.4°C, and reduced by 2.5°C at 0.05°C/a; from 1979 to the present, the MAAT varied between −1.2°C and 3.9°C, and increased rapidly by 5°C at 0.17°C/a. The variations in the calculated MAAT values showed the same tendency in a given time period (since 1949) as the annual mean temperature in China. However, the climate in the Zoigê grassland has obvious regional features because the area is located in the

![Figure 4](image-url) (a) MAAT values on a three-year scale; (b) average winter temperatures; (c) average summer temperatures from the Zoigê Weather Station [48]; (d) MAAT values calculated by MBT/CBT indices; (e) the correlation between instrumental MAAT and MAAT values calculated by MBT/CBT indices; (f) the correlation between average winter temperatures and MAAT values calculated by MBT/CBT indices; (g) the correlation between average summer temperatures and MAAT values calculated by MBT/CBT indices.
| Samples   | Depth (cm) | Concentrations of branched GDGTs (µg/g dw) | MBT | CBT |
|-----------|------------|------------------------------------------|-----|-----|
| HYS2-01   | 24.75      | 0.274, 1.812, 3.137, 0.175, 0.674, 7.649 |    |     |
| HYS2-02   | 24.25      | 0.136, 0.753, 1.404, 0.067, 0.278, 3.406 |    |     |
| HYS2-03   | 23.75      | 0.171, 0.948, 1.806, 0.092, 0.357, 4.152 |    |     |
| HYS2-04   | 23.25      | 0.276, 1.266, 2.855, 0.148, 0.613, 6.620 |    |     |
| HYS2-05   | 22.75      | 0.087, 0.444, 0.992, 0.050, 0.177, 2.195 |    |     |
| HYS2-06   | 22.25      | 0.058, 0.263, 0.583, 0.029, 0.124, 1.284 |    |     |
| HYS2-07   | 21.75      | 0.191, 0.964, 1.970, 0.099, 0.424, 4.204 |    |     |
| HYS2-08   | 21.25      | 0.260, 1.382, 2.880, 0.141, 0.606, 6.562 |    |     |
| HYS2-09   | 20.75      | 0.067, 0.314, 0.768, 0.036, 0.148, 1.741 |    |     |
| HYS2-10   | 20.25      | 0.126, 0.592, 1.326, 0.055, 0.264, 2.959 |    |     |
| HYS2-11   | 19.75      | 0.122, 0.663, 1.274, 0.072, 0.253, 3.047 |    |     |
| HYS2-12   | 19.25      | 0.052, 0.279, 0.572, 0.028, 0.125, 1.315 |    |     |
| HYS2-13   | 18.75      | 0.233, 1.067, 2.402, 0.109, 0.532, 5.556 |    |     |
| HYS2-14   | 18.25      | 0.198, 1.137, 2.287, 0.104, 0.480, 5.863 |    |     |
| HYS2-15   | 17.75      | 0.117, 0.692, 1.323, 0.059, 0.291, 3.108 |    |     |
| HYS2-16   | 16.75      | 0.142, 0.940, 1.962, 0.085, 0.457, 4.901 |    |     |
| HYS2-17   | 16.25      | 0.093, 0.557, 1.164, 0.050, 0.299, 2.897 |    |     |
| HYS2-18   | 15.75      | 0.100, 0.574, 1.192, 0.033, 0.314, 3.169 |    |     |
| HYS2-19   | 15.25      | 0.050, 0.374, 0.781, 0.030, 0.236, 1.968 |    |     |
| HYS2-20   | 14.75      | 0.065, 0.448, 0.887, 0.036, 0.280, 2.268 |    |     |
| HYS2-21   | 14.25      | 0.027, 0.175, 0.354, 0.011, 0.102, 0.862 |    |     |
| HYS2-22   | 13.75      | 0.121, 0.821, 1.575, 0.044, 0.507, 3.983 |    |     |
| HYS2-23   | 13.25      | 0.115, 0.917, 1.775, 0.044, 0.596, 4.602 |    |     |
| HYS2-24   | 12.75      | 0.063, 0.559, 1.040, 0.029, 0.329, 2.537 |    |     |
| HYS2-25   | 12.25      | 0.066, 0.565, 1.117, 0.036, 0.345, 2.969 |    |     |
| HYS2-26   | 11.75      | 0.100, 0.884, 1.710, 0.062, 0.568, 4.523 |    |     |
| HYS2-27   | 11.25      | 0.057, 0.462, 0.925, 0.026, 0.289, 2.508 |    |     |
| HYS2-28   | 10.75      | 0.117, 0.870, 1.762, 0.037, 0.580, 4.680 |    |     |
| HYS2-29   | 10.25      | 0.049, 0.407, 0.815, 0.017, 0.249, 2.282 |    |     |
| HYS2-30   | 9.75       | 0.048, 0.393, 0.913, 0.015, 0.250, 2.309 |    |     |
| HYS2-31   | 9.25       | 0.091, 0.857, 1.781, 0.029, 0.468, 4.526 |    |     |
| HYS2-32   | 8.25       | 0.116, 0.951, 2.367, 0.032, 0.615, 5.648 |    |     |
| HYS2-33   | 7.75       | 0.140, 1.301, 3.211, 0.069, 0.750, 7.784 |    |     |
| HYS2-34   | 7.25       | 0.165, 1.631, 4.049, 0.056, 1.025, 10.104 |    |     |
| HYS2-35   | 6.75       | 0.131, 1.262, 3.659, 0.033, 0.767, 9.375 |    |     |
| HYS2-36   | 6.25       | 0.053, 0.697, 2.015, 0.017, 0.412, 4.815 |    |     |
| HYS2-37   | 5.75       | 0.085, 0.977, 2.991, 0.028, 0.557, 7.731 |    |     |
| HYS2-38   | 5.25       | 0.089, 0.728, 1.627, 0.028, 0.462, 4.133 |    |     |
| HYS2-39   | 4.75       | 0.054, 0.579, 1.862, 0.012, 0.363, 5.040 |    |     |
| HYS2-40   | 4.25       | 0.036, 0.478, 1.813, 0.006, 0.314, 4.567 |    |     |
| HYS2-41   | 3.75       | 0.031, 0.444, 1.454, 0.009, 0.328, 3.701 |    |     |
| HYS2-42   | 3.25       | 0.042, 0.478, 1.502 |    |     |
| HYS2-43   | 2.75       | 0.064, 0.580, 1.765 |    |     |
| HYS2-44   | 2.25       | 0.028, 0.342, 0.948 |    |     |
| HYS2-45   | 1.75       | 0.054, 0.656, 1.815 |    |     |
| HYS2-46   | 1.25       | 0.042, 0.473, 1.197 |    |     |
| HYS2-47   | 0.75       | 0.028, 0.331, 0.896 |    |     |
| HYS2-48   | 0.25       | 0.036, 0.474, 1.090 |    |     |

a) – below the detection limit.
neat temperature of the calculated MAAT values (5.1°C) is more significant than that of the instrumental MAAT values. The variation range of the calculated MAAT values correlate better with the recorded annual mean temperatures (R² = 0.324 (P < 0.05, N = 15)) than with the average winter and summer temperatures (P < 0.05, N = 15) (Figure 4(e), (f) and (g)). However, all the correlations are relatively poor, probably because of the assumption of unvarying sedimentation rates made in the 210Pb-dating constant specific activity model, which causes the calculated time isn’t completely identical with the actual time.

The variation range of the calculated MAAT values (5.1°C) is more significant than that of the instrumental temperatures (1.4°C) in the period 1957–2001. When we examined the calibration data set of Weijers et al. [9], we found that the difference and standard deviation between the calculated and instrumental MAATs were −3.78°C, 5.95 and 3.42°C, 4.36, respectively, for instrumental MAAT < 10°C and MAAT > 10°C. This indicates that more precise empirical MAAT equations possibly need to be established for different areas, particularly on a local level, because of the different dynamic processes of soils in different areas of the world, or the different factors influencing the synthesis of branched GDGTs at different latitudes [36].

3 Conclusions

We reconstructed the variation patterns of peat pH and MAAT values in the last 150 years at the Zoigê grassland. MBT/CBT indices related to the distribution of branched GDGTs in the peat core suggest that three periods could be involved. The variation range of the calculated MAAT values (5.1°C) is more significant than that of the instrumental temperatures (1.4°C) in the last 45 years; this is probably related to the dynamic features of the local soil. The average temperature calculated by MBT/CBT is close to the average temperature recorded by the Zoigê Weather Station for the last 45 years, and the MAAT value calculated from the MBT/CBT indices shows a similar variation tendency to that obtained by tree-rings.

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