Absence of short-term temperature adaptation in core and intact branched tetraether membrane lipid distribution in a mid-latitude highland peat

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Abstract

Branched glycerol dialkyl glycerol tetraethers (GDGTs) are bacterial membrane lipids which are abundant in soils and peat and used as a proxy for temperature and pH. Several types of GDGTs exist, whose distribution can be quantified using the Methylation index of Branched tetraethers (MBT) and the Cyclisation ratio of Branched Tetraethers (CBT). These indices are used as the MBT-CBT proxy to give an indication of the annual mean air temperature (MAT). In this research, peat samples from a small bog in Moor House, Northern England were incubated for 2 months at different temperatures to see if an adaptation in the GDGT distribution could be found. To this end three different lipid types were measured using HPLC-APCI/MS: core lipids (CLs), glycolipids and phospholipids. The analysis of the different GDGTs has demonstrated that the lipid distributions, both core and intact polar lipids do not show any correlation with incubation temperatures. Instead they all reflect MAT at the sampling site. This indicates that the turnover time of the lipids is longer than the incubation time. Because of the absence of any adaptation in the tetraether membrane lipid distribution, the samples of the individual experiments were considered replicate samples and used to estimate the relative abundance of CLs, glycolipids and phospholipids in this peat. It was shown that the branched GDGTs in this peat mostly consist of CLs (85%) plus circa 10% glycolipids and 5% phospholipids. The current samples show a different lipid composition compared to those found in soils, with soils containing more phospholipids and peats more glycolipids. This might be due to differences in unknown factors during decomposition or production.

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1. Introduction

Branched glycerol dialkyl glycerol tetraethers (GDGTs) are membrane lipids which were discovered approximately a decade ago and their use as a biomarker is still being improved today. These branched GDGT lipids have octacosane alkyl units with either 13,16-dimethyl- or 5,13,16-trimethyl substitution (Schouten *et al.*, 2000; Sinninghe Damsté *et al.*, 2000). One of the methyl groups can form a cyclopentyl moiety via internal cyclisation, which is found to be the methyl group on the C-16 position (Weijers *et al.*, 2006a). Branched GDGTs have been found in high abundance in soils and peat bogs (Weijers *et al.*, 2006b; Weijers *et al.*, 2007c; Liu *et al.*, 2010). Although their exact source is still unknown, it is determined by means of the stereochemistry of the glycerol unit that they are of bacterial origin (Weijers *et al.*, 2006a). The $\delta^{13}C$ values of branched GDGT lipids and their co-variation are similar to those of bulk organic carbon and plant waxes, indicating that they might derive from heterotrophic bacteria. Weijers *et al.* (2010) have also stated, however, that the possibility of a chemoautotrophic source can not be completely excluded based on their data. Branched GDGTs have several similarities with Archaeal GDGTs, namely the membrane spanning tetraether structure and the cyclopentyl moieties. Two large structural differences exist between the branched GDGTs and isoprenoid GDGTs made by Archaea. First, the branched GDGTs comprise of branched carbon chains while Archaeal GDGTs are comprised of isoprenoid units. Second, the stereo configuration of the glycerol backbone is different, with 2,3- versus 1,2-di-*O*-alkyl-*sn*-glycerol respectively for Archaea and bacteria (Kates, 1978).

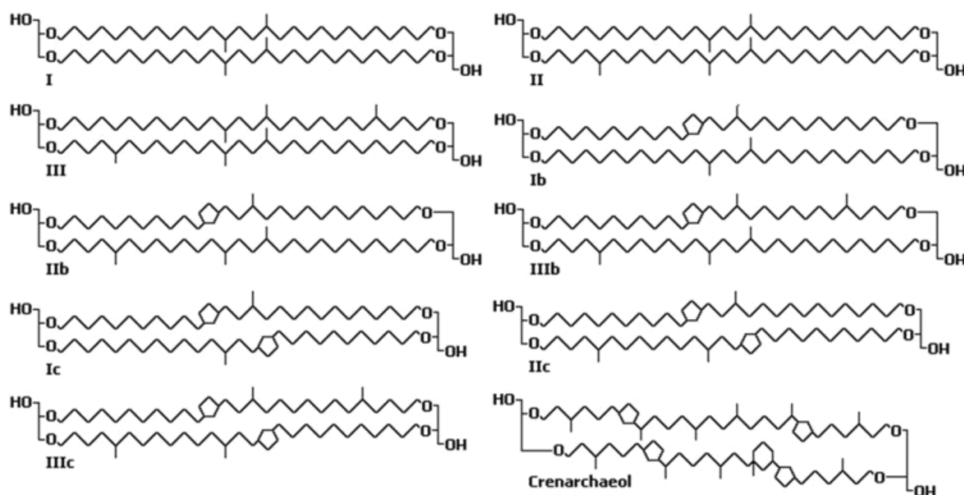

Figure 1.1: The different GDGTs discussed in this paper including crenarchaeol. The roman numerals correspond to Weijers et al *(2006) and the letters to the number of cyclopentyl moieties.*

Branched GDGTs can differ in the amount of methyl groups attached to the alkyl chain and cyclopentyl moieties formed (Sinninghe Damsté *et al.*, 2000; Weijers *et al.*, 2006a) (Fig 1.1). The number of methyl groups and cyclopentyl moieties depends largely on temperature and pH (Weijers *et al.*, 2007c). Microbes need their cell membrane to be in a liquid crystalline state and GDGT synthesizing bacteria do that by changing the amount of cyclisation and methylation in their membrane lipids. Weijers *et al.* (2007c) created two indices to express the degrees of methylation and cyclisation: the Methylation index of Branched tetraethers (MBT) and the Cyclisation ratio of Branched Tetraethers (CBT). The distribution of the GDGTs is a proxy for temperature and this distribution can be quantified with the MBT and CBT. This proxy is called the MBT-CBT proxy and gives an indication of the annual mean air temperature (Weijers *et al.*, 2007c). The MBT is a measure for the amount of methylation at the C-5 and C-5' positions of the branched GDGT. The MBT is mostly connected with temperature, though to a lesser extent also with soil pH, and is defined as follows:

$$MBT = \frac{[I + Ib + Ic]}{[I + Ib + Ic] + [II + IIb + IIc] + [III + IIIb + IIIc]} \quad (1)$$

The roman numeral plus letters correspond with figure 1.1. The CBT is mostly correlated with the pH of a sample, but it is also used for the calculation of the temperature. The CBT is defined as follows:

$$CBT = -\log\left(\frac{[Ib] + [IIb]}{[I] + [II]}\right) \quad (2)$$

The function used to calculate temperature from the MBT and the CBT has been developed by Weijers *et al.* (2007c). The temperature that is calculated via this function is mainly assumed to be the annual mean air temperature (MAT). The final formula for MAT that Weijers *et al.* (2007c) developed was:

$$MAT = \frac{(MBT - 0.122 - (0.187 * CBT))}{0.02} \quad (3)$$

Weijers *et al.* (2007c) plotted the MBT index against several other possible MATs, namely the summer, winter and month of sampling MAT. None of these MATs gave a better correlation with the MBT index than the annual MAT. Although the correlation with one of the seasonal MATs is

not better than with the annual MAT, there might still be a seasonal bias. Rueda *et al.* (2009), for example, found that the MBT-CBT derived MAT correlated best to the summer temperatures of the instrumental temperature data of the last 200 years. On the other hand, Peterse *et al.* (2009b) found that the MBT-CBT derived MAT was equal to the measured annual MAT at Svalbard despite the fact that its located on a high latitude and thus has a large seasonal change. Weijers *et al.* (2007c) found a moderate correlation between the MBT and MAT versus precipitation, but they found it doubtful that this correlation, especially between MBT and precipitation, actually exists. A final correlation that they made was between the CBT and the pH (R^2=0.70). The formula for this correlation is as follows:

$$pH = \frac{3.33 - CBT}{0.38} \qquad (4)$$

While the GDGT synthesizing bacteria live in soils and are thus independent of sunlight, they seem to depend on temperature and pH as Weijers *et al.* (2007c) found out and used in the MBT and CBT. This means that they probably also depend on nutrient input, which might be varying with the growing season of the vegetation. From the fact that these bacteria live in soils it might seem more logical that the MBT-CBT proxy gives the mean soil temperature and not the mean air temperature, but the problem is that the spatial and temporal resolution of global soil temperatures is not high enough to be used for calibration (Weijers *et al.*, 2007c). The use of air temperature instead of soil temperature has been found to explain a large part of the scatter found in the calibration of the MBT-CBT proxy (standard error of estimate of ca. 5 °C) (Weijers *et al.*, 2010). When pure cultures of these GDGT synthesizing bacteria are available, better constraints can be put on this error. The differences between air and soil temperature are mainly found to be seasonal and on a local spatial scale, while on longer temporal and larger spatial scales the MBT-CBT proxy is still thought to give proper estimates of past MAT (Weijers *et al.*, 2007a; Weijers *et al.*, in review).

Branched GDGTs can be found in different forms in soils and peat bogs. The more common and easily found form is as core lipid (CL). CLs are found in abundance in the anoxic horizons or catotelms of these soils and peat bogs (Pancost and Sinninghe Damsté, 2003; Hopmans *et al.*, 2004; Weijers *et al.*, 2006b). In these locations they generally have much higher concentrations than their Archaeal isoprenoid counterparts (Weijers *et al.*, 2009). While CLs have been studied extensively in the natural world, their precursors were much more difficult to find. The intact polar lipids (IPLs) are the true building blocks of the membranes of the bacteria that produce these lipids. The IPLs still have their polar head group (PHG), of which two types are discussed in

here (Fig 1.2), whereas the CLs are the degradation products of the IPLs after hydrolysis of the head groups (Fig. 1.1).

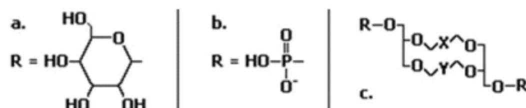

Figure 1.2: The different polar head groups, namely the glycolipid (a) and the phospholipid (b), together with the locations on the GDGT where the PHG can be found (c).

The PHG is easily lost during degradation, in comparison to the rest of the lipid. It is thus assumed that the IPLs degrade very fast to CLs when their source organism dies (White *et al.*, 1979; Harvey *et al.*, 1986; Lipp and Hinrichs, 2009). The CLs, to which the IPLs are degraded, are much more stable and can survive in sediments up to several Ma (Kuypers *et al.*, 2001). IPLs could be of importance for further study because they still have their PHGs attached, which potentially contain a lot of taxonomical and environmental information. Currently, the IPLs found in nature are presumed to be from livings cells and this means that any information gained from it would be only as old as the age of the organism that synthesized it. Weijers *et al.* (in review) have shown that IPLs might degrade slower in soils than previously thought, because they have found no seasonal temperature trend in the distribution of branched GDGT IPLs. The two different types of IPLs (Fig. 1.2) also have different degradation rates, because of the different bonds the PHG has with the glycerol part of the lipid. The phospholipids degrade much faster than the glycolipids, because they have an ester bond and an ether bond respectively (Harvey *et al.*, 1986).

Since Weijers *et al.* (in review) have found no seasonal temperature signal in soils; this might also be true for branched GDGTs in peat bogs. We looked at similar GDGTs from an English peat bog after a 2 month incubation period at several temperatures and looked for a potential change in GDGT membrane lipid distribution caused by this incubation. Although Liu *et al.* (2010) did not show the presence of phospholipids in peat bogs; we looked at the three different lipid types (CLs, phospholipids and glycolipids). Since they do not degrade at the same speed, there might be differences in the response of the lipid pools to the temperature incubations.

2. Methods

2.1 Sample collection

The *Sphagnum* moss used in the experiment has been collected from a small bog in Moor House, Northern England (54°41'N, 2°22'W) in Juli 2009. This is an area of upland blanket peat which can be up to 4 m thick (Heal *et al.*, 1975). The peat bog has a pH of 3.0 to 4.2, is oligotrophic and ombrogenous (McNamara *et al.*, 2008), thus the only source of nutrients and water is via atmospheric input. The bedrock underlying the peat contains limestone, sandstone and shale with an age of about 360-300 Ma. The climate in these moorlands has been described as sub-arctic oceanic (Heal and Perkins, 1978); with a mean monthly temperature around 10 °C and an average rainfall of 1900 mm per year (Heal *et al.*, 1975). The location was chosen because here *Sphagnum* grows in a small depression with only limited lateral water movement. The water level above the peat layer was roughly 15 cm at the collection site. This level seems to depend highly on the amount of rainfall, since during a preliminary fieldtrip in spring there was very little rainfall and the water level was only 10 cm above the peat layer. Next to the *Sphagnum* there is also an *Eriophorum* grass that grows in the depression. On higher areas surrounding the depression both *Sphagnum* and mixed grasses are the most common vegetation.

2.2 *Sphagnum* incubation

Living *Sphagnum* plus 20 cm peat was harvested, stored in coolers and brought back for incubation. The incubation was done in Rumed incubators which have a light intensity that is equal in the entire room. Each core was incubated with a different temperature, namely 5, 10, 15, 20 and 25 °C. Cores were taken with a diameter of 7 cm and a height of 50 cm. The core-tubes were transparent, but at the lower part, where the peat was located, the tubes were wrapped with aluminum foil to mimic natural conditions. The cores were incubated for 2 months until the capitula were newly grown (circa 3 cm). The water level in the cores was kept constant with bog water from the field site. After incubation the *Sphagnum* was freeze dried and stored.

2.3 Bligh Dyer extraction

The lipids were extracted from the top of the freeze dried *Sphagnum* peat material using a Bligh Dyer extraction (BDE) based on the method from Rütters *et al.* (2002). The plant material was dissolved in a BDE solvent mix of MeOH:DCM:P-buffer in the ratio 2:1:0.8 (v/v/v). The samples were extracted in an ultrasonic bath (circa 10 min) and then centrifuged to separate the supernatant from the residue (2 min at 3000 rpm). The extraction procedure was repeated two times to minimize the amount of IPLs left in the residue and every time the supernatant was collected. After the last extraction extra DCM and P-buffer was added to create a new volume

ratio of 1:1:0.9 DCM:MeOH:P-buffer, resulting in phase separation which enabled removal of the P-buffer from the extract after which the sample was centrifuged (1 min. at 3000 rpm.). The lower solvent phase (DCM-layer) was pipetted into a round bottom flask. Twice new DCM was added to the MeOH/P-buffer phase in order to remove all of the extract from the MeOH/P-buffer layer. The total of all DCM-layers in the round bottom flask is the Bligh-Dyer extract and this contains all the IPLs plus all the CLs. Most of the solvent was removed with a rotavap (waterbath at 20-25°C). The extract was then transferred to a glass vial using a DCM:MeOH (9:1) solution and further dried under N_2.

2.4 Fraction separation

The separation procedure is a modified version from the procedure used by Pitcher *et al.* (2009). It must be noted that this procedure is made and optimized for archaeal isoprenoid lipids, however, the branched GDGTs are structurally similar enough to be separated using this procedure. As preparation the silicagel was activated (3 h. at 130 °C) and put in a Pasteur pipette filled with extracted cotton wool (4 cm). The column was then rinsed with 1 column volume of solvent, after which the BDE was dissolved in a small amount of hexane:ethylacetate 1:1 (v/v) (first eluent) and transferred onto the column. The fluid was eluted with 5 column volumes of the first eluent and the material collected from the column is the CL fraction. Next, 5 column volumes of methanol (second eluent) were brought over the column and this was collected as a new fraction, which contained the IPLs. The solvent was then evaporated with N_2. The IPL fraction was divided into three different aliquots; 40% for a base hydrolysis, 40% for an acid hydrolysis and 10% for direct analysis on LC/MS to check for carry over of CLs into this IPL fraction.

2.5 Base hydrolysis of extracts

With base hydrolysis the ester-bound compounds are converted into fatty acids and alcohols. Other bonds, like glycosidic, amine and ether bonds are not affected. After the dry extract was put in a centrifuge tube and 2 ml of 1 N KOH in MeOH (96%) and a stirrer were added, it was refluxed for 1 hour. After the refluxing and the extract had cooled down the pH was adjusted to approximately 5 (based on pH indicator paper) with 2 N HCl/MeOH (1/1). Then 2 ml bidistilled water and 2 ml DCM were added and the tube was shaken to extract the organic phase (organic extracts in DCM) from the MeOH/H_2O layer. This procedure was repeated twice, but with only 1 ml DCM. The DCM with the extract was first dried over a Na_2SO_4 column to remove traces of water and further dried under a N_2-stream.

2.6 Acid hydrolysis of extracts

Acid hydrolysis converts both glycosidically-bound and ester-bound compounds into fatty acids and alcohols. A possible drawback is that the hydrolysis is an equilibrium reaction when breaking down ester bonds. To the dry extract a 1.5 N HCl/MeOH mixture was added and it was refluxed for 2 hours. When the extract was cooled down the pH was adjusted to 4-5 with 2 N KOH/MeOH 1:1 (v/v). Next, 2 ml DCM was added and the extract was shaken. After the DCM layer with extract was removed the process was repeated twice with 2 ml DCM. The combined DCM layers were put through a column of anhydrous Na_2SO_4 to dry and then further dried under N_2.

2.7 Final preparation and measurement

To all extracts C46 GDGT standard (Huguet *et al.*, 2006) was added; to the three IPL fractions 99 ng and to the CL fraction 330 ng. The extracts were then dried and 300 µl hexane/propanol 99:1 solution was added to each extract to obtain a concentration of ca. 2 mg/ml. The extracts were filtered through a 0.45 µm mesh PTFE filter, to remove all particles and dried again. Then new hexane/propanol 99:1 was added to the extracts after which they were analyzed. All samples were analyzed at Royal NIOZ. The analysis was done using high performance liquid chromatography - atmospheric pressure chemical ionization / mass spectrometry (HPLC-APCI/MS) on an Agilent 1100 series/Hewlett-Packard 1100 MSD series machine equipped with automatic injector and HP Chemstation software following Hopmans *et al.* (2000) and Schouten *et al.* (2007). The volume of the injected extract was 10 µl. Compound separation was accomplished in normal phase on an Alltech Prevail Cyano column (150 mm x 2.1 mm; 3 µm) with hexane:propanol 99:1 (v/v) as eluent (flow rate 0.2 ml min^{-1}); the first 5 min were isocratically and the next 45 min were with a linear gradient to 1.8% propanol.

2.8 Concentration and MAT calculation

Peak areas of the GDGT compounds were determined from the different mass chromatograms. With these peak areas and the peak area of the C46 standard, corrected for differences in ionization efficiency in the mass spectrometer, the concentration of each GDGT could be calculated. Next, the IPL extracts were corrected for a small carry over of CLs into the same fraction. This CL correction was applied to both the acid hydrolyzed and base hydrolyzed extracts. The base hydrolyzed extracts comprise the phospholipids and the acid hydrolyzed extracts comprise all the IPLs; so the glycolipid concentrations were calculated by deducting the base hydrolyzed extract concentrations from the acid hydrolyzed concentrations. From the concentrations of the different GDGTs the CBT ratio and MBT index could be calculated and MAT and pH could be reconstructed.

3. Results

The raw data of the samples from the five different temperatures has been reworked to the MBT and CBT ratios, the MAT and pH. In the five samples absolute concentrations of the CLs of the branched GDGTs were found to be between 3000 and 6500 ng/g total organic carbon (TOC); these CLs made up about 70-95% of the total branched GDGTs, i.e. CLs plus IPLs. The MBT and CBT ratios of the CLs show little variation between the five samples, ranging between 0.45 and 0.48 and between 1.44 and 1.56, respectively. This is also reflected when these values are translated into annual MAT and soil pH using the calibration formulas 3 and 4. This results in annual MAT estimates between 2.4 and 3.7 °C (Fig. 3.1) and soil pH estimates varying between 4.7 and 5.0.

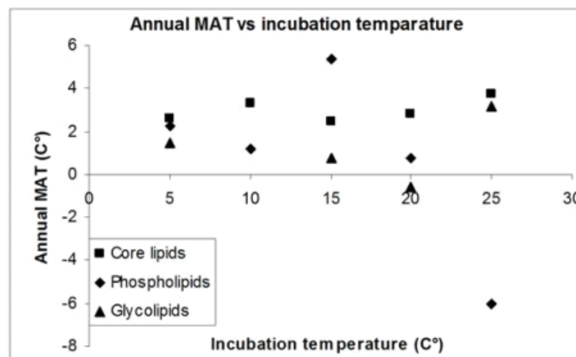

Figure 3.1: There is little change between the different incubation temperatures. The only big outlier is the phospholipid fraction of 25 °C.

Since the CLs showed no correlation with the annual MAT, the IPLs were measured as well. These IPLs degrade much faster than the CLs, so they might give a better short term signal. The concentrations of these IPLs, although much lower than the CLs, were still significant, namely between 350 and 1250 ng/g TOC (6 to 29% of total branched GDGTs). The MBT and CBT ratios values do not differ much from the CL values, 0.39 to 0.46 and 1.50 to 1.63 respectively, which translates into a MAT of -1.2 to 1.8 °C and a pH of 4.5 to 4.8.

Using different hydrolysis methods (base and acid hydrolysis), two different types of IPLs were determined in the IPL fraction, namely the glycolipids and phospholipids. The glycolipid concentrations range between 350 and 1150 ng/g TOC and the phospholipid concentrations range between 50 and 1150 ng/g TOC. The MBT and CBT ratios of the two different IPLs are again rather constant, although the range is somewhat larger than based on the total IPLs,

especially for the phospholipids. The MBT and CBT ratios of the glycolipids range between 0.41 and 0.56 and between 1.51 and 1.68 and for the glycolipids between 0.40 and 0.50 and between 1.35 and 1.58. This translates into a MAT for the glycolipids of -0.6 to 3.2 °C and for the phospholipids -6.0 to 5.4 °C (Fig. 3.1) and a pH for the glycolipids of 4.3 to 4.8 and the phospholipids of 4.6 to 5.2.

4. Discussion

After the cores have been incubated for 2 months at different temperatures, i.e. 5, 10, 15, 20 and 25 °C, it was expected that this incubation would have resulted in some changes in the distribution of branched GDGTs and thus in MBT/CBT estimated temperatures. However, for the CLs no correlation could be found with the incubation temperatures (Fig. 3.1). The calculated MAT is constant around 3.0 ± 0.5 °C and is actually much more similar to the annual MAT of the field site than to the incubation temperatures. The time over which the MAT as recorded by the membrane lipid distribution is smoothed depends highly on the turnover time of the lipids. Earlier studies have found that the turnover time of CLs of branched GDGTs is much longer than the two months the samples were incubated. Weijers *et al.* (2010) showed via stable carbon isotope composition of branched alkanes (released from branched GDGTs) that their CLs have a turnover time of 20 years. Also, Peterse *et al.* (2010) showed that the CL composition of branched GDGTs needed up to 40 years to adjust fully to a manipulated change in pH in a grassland soil. So, it is not surprising that the signal found is not of the incubation temperatures, but the field temperature.

Since IPLs are assumed to better reflect living biomass rather than CLs, which represent a circa 20 year average, IPLs have been analyzed to see if they reflect some influence of the two month incubation. Although this assumption is probably right, the IPLs do not show any signal of the incubation (Fig. 3.1). Just like the CLs they only show the field temperature, namely 0.4 ± 1.3 °C. Thus, this indicates that also the turnover time of the IPLs is probably longer than two months. Weijers *et al.* (in review) indeed suggest that the turnover time of IPLs might even be in the order of one year, which means that the incubation of two months has been too short to sort any effect in the distribution of the branched GDGTs that were already present in the samples prior to incubation.

In the methods (Section 2.8) it is shown that several corrections were applied to the initially calculated concentrations. One of these steps was the correction for carryover of CLs into the IPL fractions. Although the amount of CLs that is actually found in the IPL fractions is low compared to the total amount of CLs (around 3% of the total amount of CLs in the sample), it could make up a considerable portion of the IPL fraction (6-36%). A problem with this correction is that there is a large uncertainty introduced during the preparation of these extracts. When the total IPL fraction is divided in the three different aliquots, the DCM that is used as solvent is evaporating quickly. This means that the division into 40:40:20 is not always very accurate and the theoretical 20% of the solution that is used as a CL-check could in practice be as low as 10% of

the solution. This would mean that the actual concentrations are twice as high as the concentrations used here for the CL correction. Another part of the error could be the pipette that has been used. These pipettes can add errors as large as 10%. Thus, the error that is subsequently introduced into the calculations could be substantial. The error caused with this correction is quite possibly even larger than the one removed with it. On the other hand the relative change between the two different lipid types does not change much if the correction is not applied (Fig 4.2 and 4.3).

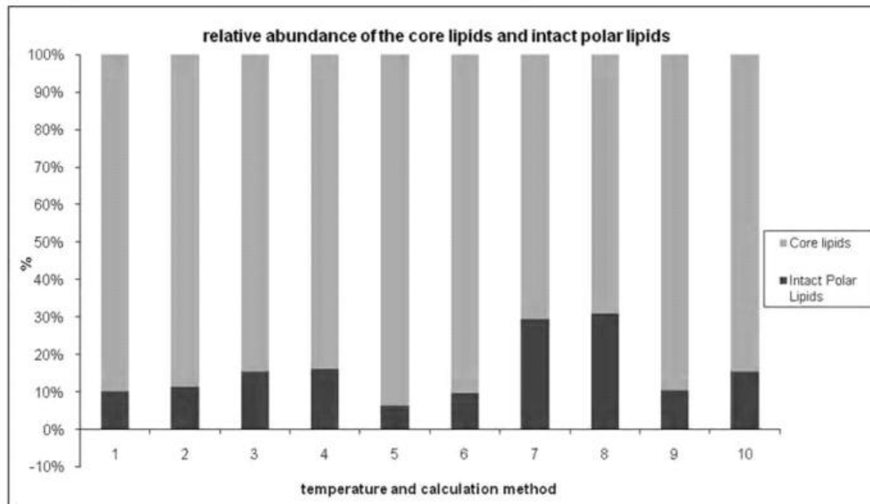

Figure 4.2: The two different calculation methods are the original method from the method section with CL-correction (a) and the method without the CL-correction (b).

IPLs consist of two types, namely glycolipids and phospholipids. These different IPLs were measured separately to see if phospholipids, which are assumed to be more labile, might better reflect the incubation temperatures than the total IPL pool. The IPL pool also contains glycolipids which are more stable than phospholipids and might behave more like CLs. Especially since Liu *et al* (2010) suggested that the vast majority of IPLs in peat consists of glycolipids. The glycolipids show a larger standard deviation but a similar temperature as the total IPLs, namely 1.2 ± 1.6 °C (Fig. 3.1). It must be noted that for the glycolipids only 4 temperatures were used, because some of the concentrations of the branched GDGTs for 10 °C were negative and a calculation of the MAT was impossible. It is not surprising to see that this MAT does not differ much from the MAT of the CLs and the total IPLs, since the glycolipids are the more stable IPLs.

Although the phospholipids are the most labile of all lipid types and thus most represent living biomass, they still do not show any MAT change towards the incubation temperatures. The phospholipids have the lowest calculated MAT of 0.7 ± 4.2 °C, but some concentrations of the phospholipid GDGTs, namely from 15 °C and 25 °C, were negative and thus their calculated MAT values (although it was possible to compute one) could not be trusted and probably give rise to the larger standard deviation. A good reason not to apply the CL correction on the IPLs is that these negative concentrations are removed. This makes the data more useful, because almost all concentrations can be properly used. The standard deviations of both the glycolipids (1.2 ± 1.6°C) and the phospholipids (2.6 ± 1.0 °C) are now much better. There is a slight disadvantage when this correction is not applied, namely that the CLs in the IPL fraction could cause this fraction to deviate towards the CL distributions and thus CL MAT. The deviation is small and does not cause much shift in the data (Fig. 4.2).

These data show that the lipids, whether they are CLs or IPLs, might not have a temperature signal of the last few months, but much longer (Fig. 3.1). There could be several reasons that not only the CLs but also the glyco- and phospholipids have smoothing effects on timescales of more than a couple of months. The bacteria that are synthesizing these GDGTs might grow slowly, e.g. several Acidobacteria, who might be the branched GDGT synthesizing bacteria (Weijers *et al.*, 2009), are slow growers (Eichorst *et al.*, 2007). Another possibility is that the lipids have slower degradation rates than earlier suspected. While it was already known of CLs that their degradation could take up to 20-40 years, for IPLs this is rather new. These two reasons are probably both partly causing the long turnover times of IPLs. These are also the most probable reason that there is no trend seen between the five different temperatures. Because of this, the five experiments might, then, as well be treated as as replicates samples to look at the proportions of the different branched GDGT lipids fractions in the peat .

Another problem, which is addressed above, is the 10 °C sample, in which the phospholipid extract has similar concentrations as the total IPL extract. This would mean that in this sample there are almost no glycolipids, which is in contradiction with the other samples because they all have more glycolipids than phospholipids (Fig 4.2). It is also in contradiction with Liu *et al.* (2010) who only found glycolipids in peat bogs. It might be possible that by mistake the phospholipid extract is treated as a total lipid extract and thus all the IPLs in the extract have been measured. It will thus be assumed that this sample gives twice the total IPL fraction. With only the total IPL fraction and no way to determine the concentration of the two different IPL fractions, the 10 °C sample has been left out and the relative abundances have been recalculated based on four samples. Especially with the removal of the 10 °C sample the relative abundances

change (Fig 4.3). Now the glycolipids are more abundant than the phospholipids (10.9% and 4.5% of the total lipid fraction respectively), where earlier these were present in approximately equal amounts. This seems more in line with results presented Liu *et al.* (2010), namely that glycolipid branched GDGTs dominate in a peat bog. This is different from soils, because in soils the phospholipids represent the majority of the IPLs (Weijers *et al.*, in review). This probably means that the GDGT producing microbial communities in soils are different from those found in peats.

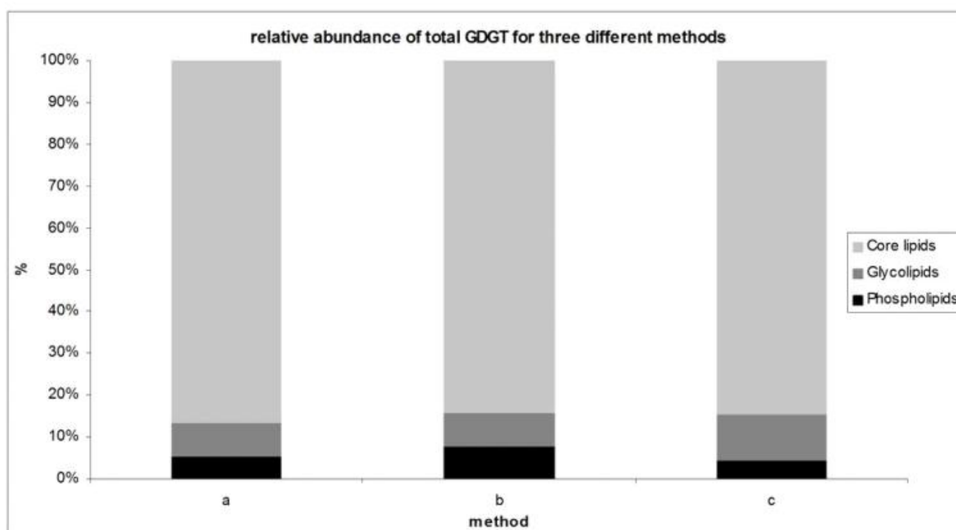

Figure 4.3: The three different calculation methods are the original method from the method section with CL-correction (a), the method without the CL-correction (b) and the method where the 10 °C sample is excluded (c).

The ratio between phospho- and glycolipids might indicate that the microbial degradation rates of these lipids are much higher in peats. When the degradation rates are higher the relative amounts of glycolipids versus phospholipids will also be higher, because of the faster degradation of phospholipids. Assuming that peat contains more organic matter than other soils, this is in contradiction with Harvey *et al.* (1986), who found that the degradation rate of phospholipids is lower relative to the glycolipids when there is more organic matter present. Therefore, it is more likely that branched GDGT producers in peat synthesize IPLs with different ratios phospholipids vs. glycolipids then in soils. This might have to do with limited phosphate availability in peat compared to soils (Bedford *et al.*, 1999) or other physical and/or chemical characteristics of peat might play a role in this.

5. Conclusions

The analysis of different branched GDGTs of bacterial membrane lipids has shown that these lipids do not show any correlation with incubation temperatures. This is shown for CLs, total IPLs and the different types of IPLs, i.e. glycolipids and phospholipids, indicating that the turnover time of all these lipid types is longer than the two months they have been incubated. The lack of a visible incubation signal in the calculated MAT suggests even significantly longer turnover times, in concordance with reported values of at least 20 years for CLs (Weijers *et al.,* 2010; Peterse *et al.,* 2010) and of approximately one year for IPLs (Weijers *et al.,* in review).

A few changes were made to the initial method of the GDGT calculations. First, the CL-correction was removed, which corrects for the CLs that are carried over to the IPL fraction. When this correction was applied several concentrations became negative, so by omitting this correction these negative values were removed. Also, the error that is implemented by applying the CL-correction might be larger than the error caused by the CLs in the IPL fraction. After eliminating this correction there was still no correlation between incubation temperature and calculated MAT from the different lipid types. One should always apply the CL-correction, but also realize that the errors caused by this correction could be large. The correction should thus always be extensively checked for its errors, especially during preparation.

No correlation was found between the incubation temperatures and the calculated MATs, therefore the different samples for each lipid type can be considered as replicates. As second correction the 10°C sample was removed, which is a sample that was probably not handled correctly during preparation since the phospholipid fraction has probably been treated as a total IPL fraction. It was then shown that the branched GDGTs in peat mostly consist of CLs plus circa 10% glycolipids and circa 5% phospholipids, where the glycolipids and the phospholipids had much more similar concentrations when the 10°C sample was still included.

The samples show a different composition than those found in soils. Soils contain much more phospholipids than glycolipids, where peats have much more glycolipids (Weijers *et al.*, in review). This might be the case, because the decomposition in peats depends partly on other factors than in soils. What these factors might be is still mostly unknown and should be further investigated although P availability might be an important factor. It is also unknown which bacteria synthesize branched GDGTs, thus finding them might give important information as well. In the future cultures of these bacteria should be used improve our knowledge on the effect of growing temperatures and degradation rates on the composition of their membrane lipids.

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