Ubiquitous production of branched glycerol dialkyl glycerol tetraethers (brGDGTs) in global marine environments: a new source indicator for brGDGTs

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Abstract. Presumed source specificity of branched glycerol dialkyl glycerol tetraethers (brGDGTs) from bacteria thriving in soil/peat and isoprenoid GDGTs (iGDGTs) from aquatic organisms led to the development of several biomarker proxies for biogeochemical cycle and paleoenvironment. However, recent studies reveal that brGDGTs are also produced in aquatic environments besides soils and peat. Here we examine three cores from the Bohai Sea and found distinct difference in brGDGT compositions varying with the distance from the Yellow River mouth. We thus propose an abundance ratio of hexamethylated to pentamethylated brGDGT (IIIa/IIa) to evaluate brGDGT sources. The compilation of globally distributed 1354 marine sediments and 589 soils shows that the IIIa/IIa ratio is generally <0.59 in soils, 0.59–0.92 and >0.92 in marine sediments with and without significant terrestrial inputs, respectively. Such disparity confirms the existence of two sources for brGDGTs, a terrestrial origin with lower IIIa/IIa and a marine origin with higher IIIa/IIa, which is likely attributed to generally higher pH and the production of brGDGTs in cold deep

water in sea. The application of the IIIa/IIa ratio to the East Siberian Arctic Shelf proves it a sensitive source indicator for brGDGTs, which is helpful for accurate estimation of organic carbon source and paleoclimates in marine settings.

1 Introduction

Glycerol dialkyl glycerol tetraethers (GDGTs), membrane lipids of archaea and certain bacteria, are widely distributed in marine and terrestrial environments (Reviewed by Schouten et al., 2013). These lipids have been a focus of attention of organic geochemists for more than ten years because they can be used to estimate environmental variables in the past such as temperature, soil pH, organic carbon source and microbial community structure (e.g., Schouten et al., 2002; Hopmans et al., 2004; Weijers et al., 2006; Lipp et al., 2008; Kim et al., 2010; Peterse et al., 2012; Zhu et al., 2016). There are generally two types of GDGTs, isoprenoid (iGDGTs) and non-isoprenoid, branched GDGTs (brGDGTs; Fig. 1). The former group is more abundant in aquatic settings and generally thought to be produced by Thaumarchaeota, a specific genetic cluster of the archaea domain (Sinninghe Damsté et al., 2002; Schouten et al., 2008), although Euryarchaeota may be a significant source of iGDGTs in the ocean (e.g., Lincoln et al., 2014). In contrast, the 1,2-di-*O*-alkyl-*sn*-glycerol configuration of brGDGTs was interpreted as an evidence for a bacterial rather than archaeal origin for brGDGTs (Sinninghe Damsté et al., 2000; Weijers et al., 2006). So far, only one brGDGT with two 13,16-dimethyl octacosanyl moieties was unambiguously detected in two species of Acidobacteria (Sinninghe Damsté et al., 2011), which hardly explains high diversity and ubiquitous occurrence of up to 15 brGDGT isomers in environments (Weijers et al., 2007b; De Jonge et al., 2014). Therefore, other biological sources of brGDGTs, although not yet identified, are likely.

The source difference between brGDGTs and iGDGTs led researchers to developing a branched and isoprenoid tetraether (BIT) index, expressed as relative abundance of terrestrial-derived brGDGTs to aquatic-derived Thaumarchaeota (Hopmans et al., 2004). Subsequent studies found that the BIT index is specific for soil

organic carbon because GDGTs are absent in vegetation (e.g., Walsh et al., 2008; Sparkes et al., 2015). The BIT index is generally higher than 0.9 in soils, but close to 0 in marine sediments devoid of terrestrial inputs (Weijers et al., 2006; Weijers et al., 2014). Since its advent, the BIT index has been increasingly used to trace soil organic matter in different environments (e.g., Herfort et al., 2006; Kim et al., 2006; Blaga et al., 2011; Loomis et al., 2011; Wu et al., 2013). However, the BIT index is not just dependent on the abundance of brGDGTs, which reflects the input of soil organic matter, but also on the abundance of crenarchaeol, which is linked to marine productivity (e.g., Herfort et al., 2006; Smith et al, 2012; Fietz et al., 2012). Besides the BIT index, Weijers et al. (2007b) found that the number of cyclopentane moieties of brGDGTs, expressed as Cyclization of Branched Tetraethers (CBT), correlated negatively with soil pH, while the number of methyl branches of brGDGTs, expressed as Methylation of Branched Tetraethers (MBT), was dependent on annual mean air temperature (MAT) and to a lesser extent on soil pH. The MBT/CBT proxies were further corroborated by subsequent studies (e.g., Sinninghe Damsté et al., 2008; Peterse et al., 2012; Yang et al., 2014a). Assuming that brGDGTs preserved in marine sediments close to the Congo River outflow were derived from soils in the river catchment, Weijers et al. (2007a) reconstructed large-scale continental temperature changes in tropical Africa that span the past 25,000 years by using the MBT/CBT proxy. Recently, De Jonge et al. (2013) used a tandem high performance liquid chromatography-mass spectrometry (2D HPLC-MS) and identified a series of novel 6-methyl brGDGTs which were previously coeluted with 5-methyl brGDGTs. This finding resulted in the redefinition and recalibration of brGDGTs' indexes (e.g., De Jonge et al., 2014; Xiao et al., 2015).

One underlying assumption of all brGDGT-based parameters is their source specificity, i.e., brGDGTs is only biosynthesized by bacteria thriving in soils and peat. Several studies, however, observed different brGDGT compositions between marine sediments and soils on adjacent lands, supporting in situ production of brGDGTs in marine environments (e.g., Peterse et al., 2009a; Zhu et al., 2011; Liu et al., 2014; Weijers et al., 2014; Zell et al., 2014), analogous to lacustrine settings (e.g., Sinninghe Damsté et al., 2009; Tierney & Russell, 2009; Tierney et al., 2012) and rivers (e.g., Zhu

et al., 2011; De Jonge et al., 2015; French et al., 2015; Zell et al., 2015). Peterse et al. (2009) compared the brGDGTs' distribution in Svalbard soils and nearby fjord sediments, and found that concentrations of brGDGTs (0.01–0.20 μg/g dw) in fjord sediments increased towards the open ocean and the distribution was strikingly different from that in soil. Zhu et al. (2011) examined distributions of GDGTs in surface sediments across a Yangtze River-dominated continental margin, and found evidence for production of brGDGTs in the oxic East China Sea shelf water column and the anoxic sediments/waters of the Lower Yangtze River. At the global scale, Fietz et al. (2012) reported a significant correlation between concentrations of brGDGTs and crenarchaeol ($p < 0.01$; $R^2 = 0.57–0.99$), suggesting that a common or mixed source for brGDGTs and iGDGTs are actually commonplace in lacustrine and marine settings. More recently, Sinninghe Damsté (2016) reported tetraethers in surface sediments from 43 stations in the Berau River delta (Kalimantan, Indonesia), and this result, combined with data from other shelf systems, are coherent with the hypothesis that brGDGTs are in situ produced in shelf sediments especially at water depth of 50–300 m.

Fluvial inputs and wind are the most important pathways for transporting terrestrial material into sea. In continental shelf, fluvial discharge is more important because brGDGTs in atmospheric dust are either below the detection level (Hopmans et al., 2004) or present at low abundance (Fietz et al., 2013; Weijers et al., 2014). In the remote ocean where no direct impact from land erosion via rivers takes place, eolian transport and in situ production are major contributors for brGDGTs. Weijers et al. (2014) found that distributions of African dust-derived brGDGTs were similar to those of soils but different from those of distal marine sediments, providing a possibility to distinguish terrestrial vs. marine brGDGTs based on molecular compositions. However, so far no robust molecular indicator is available for estimating source of brGDGTs in marine environments. Considering this, we conduct a detailed study about GDGTs in three cores from the Bohai Sea which are subject to the Yellow River influence to different degree. Our purpose is to evaluate the source discerning capability of different brGDGT parameters, from which the most sensitive parameter is selected and applied

for globally distributed marine sediments and soils to test whether it is valid at the global scale. Our study supplies an important step for improving accuracy of brGDGT-derived proxies and better understanding marine carbon cycle and paleoenvironments.

2 Material and methods

2.1 Study area and sampling

The Bohai Sea is a semi-enclosed shallow sea in northern China, extending about 550 km from north to south and about 350 km from east to west. Its area is 77,000 km^2 and the mean depth is 18 m (Hu et al., 2009). The Bohai Strait at the eastern portion is the only passage connecting the Bohai Sea to the outer Yellow Sea. Several rivers, including Yellow River, the second largest river in the world in terms of sediment load (Milliman & Meade, 1983), drain into the Bohai Sea with a total annual runoff of 890×10^8 m^3. A 64 cm long gravity core (M1; 37.52°N, 119.32°E) was collected in July 2011, while other two cores, M3 (38.66°N, 119.54°E; 53 cm long) and M7 (39.53°N, 120.46°E; 60 cm long), were collected in July 2013 (Fig. 2). The sites M1, M3 and M7 are located in the south, the center and the north of the Bohai Sea, respectively. The cores were transported to the lab where they were sectioned at 1 or 2 cm interval. The age model was established on basis of ^{210}Pb and ^{137}Cs activity, showing that the bottom sediments are less than 100 years old (Wu et al., 2013 and unpublished data).

2.2 Lipid extraction and analyses

The detailed procedures for lipid extraction and GDGT analyses have been described in previous studies (Ding et al., 2015; Xiao et al., 2015). Briefly, the homogenous freeze-dried samples were ultrasonically extracted with dichloromethane (DCM)/methanol (3:1 v:v). The extracts were separated into nonpolar and polar fraction over silica gel columns. The latter fraction containing GDGTs was analyzed using an Agilent 1200 HPLC-atmospheric pressure chemical ionization-triple quadruple mass spectrometry (HPLC-APCI-MS) system. The separation of 5- and 6-methyl brGDGTs was achieved with two silica columns in sequence (150 mm×2.1 mm; 1.9 μm, Thermo Finnigan; USA). The quantification was achieved by comparison of the respective

146 protonated ion peak areas of each GDGT to the internal standard (C_{46} GDGT) in
147 selected ion monitoring (SIM) mode. The protonated ions were m/z 1050, 1048, 1046,
148 1036, 1034, 1032, 1022, 1020, 1018 for brGDGTs, 1302, 1300, 1298, 1296, 1292 for
149 iGDGTs and 744 for C_{46} GDGT.
150
151 2.3 Parameter calculation and statistics
152 The BIT, MBT, Methyl Index (MI), Degree of Cyclization (DC) of brGDGTs and
153 weighted average number of cyclopentane moieties for tetramethylated brGDGTs
154 ($\#Rings_{tetra}$) were calculated according to the definitions of Hopmans et al. (2004),
155 Weijers et al. (2007b), Zhang et al. (2011), Sinninghe Damsté et al. (2009) and
156 Sinninghe Damsté (2016), respectively.

157 $$BIT = \frac{Ia + IIa + IIIa}{Ia + IIa + IIIa + IV} \quad (1)$$

158 $$MBT = \frac{Ia + Ib + Ic}{Ia + IIa + IIIa + Ib + IIb + IIIb + Ic + IIc + IIIc} \quad (2)$$

159 $$MI = 4 \times (Ia + Ib + Ic) + 5 \times (IIa + IIb + IIb) + 6 \times (IIIa + IIIb + IIIc) \quad (3)$$

160 $$DC = \frac{Ib + IIb}{Ia + IIa + Ib + IIb} \quad (4)$$

161 $$\#Rings_{tetra} = \frac{Ib + 2 \times Ic}{Ia + Ib + Ic} \quad (5)$$

162 where roman numbers denote relative abundance of compounds depicted in Fig. 1. In
163 this study, we used two silica LC columns in tandem and successfully separated 5- and
164 6-methyl brGDGTs. However, many previous studies (e.g., Weijers et al., 2006) used
165 one LC column and did not separate 5- and 6-methyl brGDGTs. Considering this, we
166 combined 5-methyl and 6-methyl brGDGT as one compound in this study, for example,
167 IIIa denotes the total abundance of brGDGT IIIa and IIIa' in figure 1.
168 An analysis of variance (ANOVA) was conducted for different types of samples
169 to determine if they differ significantly from each other. The SPSS 16.0 software
170 package (IBM, USA) was used for the statistical analysis. Squared Pearson correlation
171 coefficients (R^2) were reported and a significance level is $p < 0.05$.
172
173 2.4 Data compilation of global soils and marine sediments

The dataset in this study are composed of relative abundance of GDGTs and derived parameters from 1354 globally distributed soils and 589 marine sediments (Fig. 2 and supplementary data). These sampling sites span a wide area from 75.00°S to 79.28°N and 168.08°W to 174.40°E and the water depth ranges from 1.0 to 5521 m. The marine samples are from the South China Sea (Hu et al., 2012; Jia et al., 2012; O'Brien et al., 2014; Dong et al., 2015), Caribbean Sea (O'Brien et al., 2014), western equatorial Pacific Ocean (O'Brien et al., 2014), southeast Pacific Ocean (Kaiser et al., 2015), the Chukchi and Alaskan Beaufort Seas (Belicka & Harvey, 2009), eastern Indian Ocean (Chen et al., 2014), East Siberian Arctic Shelf (Sparkes et al., 2015), Kara Sea (De Jonge et al., 2015; De Jonge et al., 2016), Svalbard fjord (Peterse et al., 2009a), Red Sea (Trommer et al., 2009), the southern Adriatic Sea (Leider et al., 2010), Columbia estuary (French et al., 2015), globally distributed distal marine sediments (Weijers et al., 2014) and the Bohai Sea (this study). Soil samples are from the Svalbard (Peterse et al., 2009b), Columbia (French et al., 2015), China (Yang et al., 2013; Yang et al., 2014a; Yang et al., 2014b; Ding et al., 2015; Xiao et al., 2015; Hu et al., 2016), globally distributed soils (Weijers et al., 2006; Peterse et al., 2012; De Jonge et al., 2014), California geothermal (Peterse et al., 2009b), France and Brazil (Huguet et al., 2010), western Uganda (Loomis et al., 2011), the USA (Tierney et al., 2012), Tanzania (Coffinet et al., 2014), Indonesian, Vietnamese, Philippine, China and Italia (Mueller-Niggemann et al., 2016).

3 Results and discussion

3.1 Distribution and source of brGDGTs in Bohai Sea

A series of iGDGTs including crenarchaeol and brGDGTs including 5-methyl and 6-methyl isomers were detected in Bohai Sea sediments. For brGDGTs, a total of 15 compounds were identified including three tetramethylated brGDGTs (Ia, Ib and Ic), six pentamethylated brGDGTs (IIa, IIb, IIc, IIa', IIb' and IIc') and six hexamethylated brGDGTs (IIIa, IIIb, IIIc, IIIa', IIIb' and IIIc'). In order to evaluate provenances of brGDGTs, we calculated various parameters including the BIT index, percentages of tetra-, penta- and hexa-methylated brGDGTs, #rings for tetramethylated brGDGTs, DC,

MI, MBT, brGDGTs IIIa/IIa and Ia/IIa (Table 1). The value of the BIT index ranged from 0.27 to 0.76 in the core M1, which is much higher than that in the core M3 (0.04–0.25) and the core M7 (0.04–0.18). Such difference is not surprising since the site M1 is closest to the Yellow River outflow, and receives more terrestrial organic carbon than other two sites (Fig. 2). However, the BIT index itself has no ability to determine the source of brGDGTs (terrestrial vs. aquatic) because brGDGTs and crenarchaeol used in this index are thought to be specific for soil organic carbon and marine organic carbon, respectively (Hopmans et al., 2004), although crenarchaeol is also present in soils at low abundance (Weijers et al., 2006). For individual brGDGTs, the core M1 is characterized by significantly higher percentage of brGDGT IIa (28±1%) than the core M2 (18±1%) and the core M3 (18±0%; Fig. 3). We performed ANOVA for a variety of brGDGTs' parameters. All results except from MI show a significant difference between Chinese soils and Bohai Sea sediments. The IIIa/IIa ratio is the most sensitive parameter which can completely separate the samples into four groups: Chinese soils (0.39±0.25; Mean±SD; same hereafter), M1 sediments (0.63±0.06), M3 sediments (1.16±0.12) and M7 sediments (0.93±0.07).

Three factors may account for the occurrence of higher IIIa/IIa ratio in the Bohai Sea sediments than Chinese soils: selective degradation during land to sea transport, admixture of river produced brGDGTs and in situ production of brGDGTs in sea. Huguet et al. (2008; 2009) reported that iGDGTs (i.e., crenarchaeol) was degraded at a rate of 2-fold higher than soil derived brGDGTs under long term oxygen exposure in the Madeira Abyssal Plain, leading to increase of the BIT index. Such selective degradation, however, cannot explain significant different IIIa/IIa ratio between the Chinese soils and Bohai Sea sediments because unlike crenarchaeol, both IIIa and IIa belong to brGDGTs with similar chemical structures and thus have similar degradation rates. In situ production of brGDGTs in rivers is a widespread phenomenon, and can change brGDGTs' composition in sea when they were transported there (e.g., Zhu et al., 2011; De Jonge et al., 2015; Zell et al., 2015). However, this effect is minor in the Yellow River because extremely high turbidity (up to 220 kg/m^3 during the flood season; Ren & Shi, 1986) greatly constrain the growth of aquatic organisms. The studies along

lower Yellow River-estuary-coast transect suggested that brGDGTs in surface sediments were primarily a land origin (Wu et al., 2014). In our study, the site M1 is adjacent to the Yellow River mouth and receives the largest amount of terrestrial organic matter, causing lower IIIa/IIa values (0.63±0.06). In contrast, the site M3 located in central Bohai Sea comprises of the least amount of terrestrial organic matter, resulting in higher IIIa/IIa values (1.16±0.12). The intermediate IIIa/IIa value at the site M7 (0.93±0.07) is attributed to moderate land erosion nearby northern Bohai Sea (Fig. 2). These results, consistent with other terrestrial biomarkers such as C_{29} and C_{31} *n*-alkanes and C_{29} sterol (unpublished data), suggest that the higher IIIa/IIa values in the Bohai Sea sediments compared to Chinese soils (0.39±0.25) is most likely caused by in situ production of brGDGTs.

3.2 Regional and global validation of brGDGT IIIa/IIa

To test whether the IIIa/IIa ratio is valid in other environments, we apply it to the Svalbard (Peterse et al., 2009a), the Yenisei River outflow (De Jonge et al., 2015) and the East Siberian Arctic Shelf (Sparkes et al., 2015). Similar to Bohai Sea, the brGDGT IIIa and IIa are also ubiquitously present in these environments. By comparing the compositions of brGDGTs in Svalbard soils and nearby fjord sediments, Peterse et al. (2009a) indicated that sedimentary organic matter in fjords was predominantly a marine origin. A plot of BIT vs. IIIa/IIa (Fig. 4a) clearly grouped the samples into two groups which correspond to soils (>0.75 for BIT and <1.0 for IIIa/IIa) and marine sediments (<0.3 for BIT and >1.0 for IIIa/IIa). Another line of evidence is from De Jonge et al. (2015) who examined brGDGTs in core lipids (CLs) and intact polar lipids (IPLs) in the Yenisei River outflow. As the IPLs are rapidly degraded in the environment, they can be used to trace living or recently living material, while the CLs are generated via degradation of the IPLs after cell death (White et al., 1979; Lipp et al., 2008). The compilation of brGDGTs' abundance from De Jonge et al. (2015) shows significant difference of the IIIa/IIa ratio between the IPL fractions (>1.0) and CL fractions (<0.8; Fig. 4b). Such disparity supports that brGDGTs produced in marine environments have higher IIIa/IIa values because labile intact polar brGDGTs are mainly produced in situ,

whereas recalcitrant core brGDGTs are composed of more allochthonous terrestrial components. Sparkes et al. (2015) examined brGDGTs in surface sediments across the East Siberian Arctic Shelf (ESAS) including the Dmitry-Laptev Strait, Buor-Khaya Bay, ESAS nearshore and ESAS offshore. The plot of BIT vs. IIIa/IIa again results into two groups, one group with lower BIT values (<0.3) and higher IIIa/IIa values (0.8–2.3) mainly from ESAS offshore, and another group with higher BIT values (0.3–1.0) and lower IIIa/IIa values (0.4–0.9) from the Dmitry-Laptev Strait, Buor-Khaya Bay and ESAS nearshore (Fig. 4c). A strong linear correlation was observed between the IIIa/IIa ratio and the distance from river mouth (R^2=0.58; p<0.05; Fig. 4d), in accord with the data of the BIT index and $\delta^{13}C_{org}$ (Sparkes et al., 2015). All lines of evidence support that marine-derived brGDGTs have higher IIIa/IIa values than terrestrial derived brGDGTs.

We further extend the dataset on global scale (Fig. 5), showing that the IIIa/IIa ratio is still significantly higher in marine sediments than soils ($p < 0.01$). An exception was observed for Red Sea sediments which have unusually low IIIa/IIa values (0.39±0.21) compared to other marine sediments (>0.87). The Red Sea has a restricted connection to the Indian Ocean via the Bab el Mandeb Strait. This, combined with high insolation, low precipitation and strong winds result in surface water salinity up to 41 in the south and 36 in the north of the Red Sea (Sofianos et al., 2002). Under such extreme environment, distinct microbial populations may be developed and produced GDGTs different from that in other marine settings (See Trommer et al., 2009 for details).

Overall, the global distribution of IIIa/IIa presents the highest values in many deep sea sediments (2.6–5.1), the lowest values in soils (<1.0), and intermediate values in sediments from bays, coastal areas or marginal seas (0.87–2.62; Fig. 5). These results are consistent with our data from the Bohai Sea, and confirm that the IIIa/IIa ratio is a useful proxy for tracing the source of brGDGTs in marine sediments at regional and global scales.

Why do marine sediments generally have higher IIIa/IIa values than soils? It has been reported that relative number of methyl groups positively correlates with soil pH

and negatively correlates with MAT (Weijers et al., 2007b; Peterse et al., 2012). The IIIa/IIa ratio is actually an abundance ratio of hexamethylated to pentamethylated brGDGT, and thus is also affected by ambient temperature and pH. Unlike iGDGTs which is well known to be mainly produced by Thaumarchaeota (Sinninghe Damsté et al., 2002; Schouten et al., 2008), the marine source of brGDGTs remains elusive. Here, we assume that marine organisms producing brGDGTs response to ambient temperature in the same way as those soil bacteria producing brGDGTs, i.e., a negative correlation between relative number of methyl group of brGDGTs and ambient temperature. Because a large temperature gradient exists from surface to bottom water in ocean, we need consider the locale where brGDGTs are produced. If brGDGTs in marine environments are predominantly produced in euphotic zone, we would not observe a significant difference for the IIIa/IIa ratio between land and sea because both soils and marine sediments are globally distributed, leading to no systematic difference between soil temperature and sea surface temperature. Alternatively, if brGDGTs in marine sediments are partially derived from deep-water dwelling or benthic organisms, cold deep water (generally 1–2 ^{o}C) would cause higher IIIa/IIa values in marine sediments, as we observed in this study. Although to the best of our knowledge, there is no study reporting in situ production of brGDGTs throughout water column in ocean. Recent studies (Taylor et al., 2013; Kim et al., 2015) have suggested that Thaumarchaeota thriving in the deeper, bathypelagic water-column (>1000 m water depth) biosynthesized iGDGTs with different compositions as surface dwelling Thaumarchaeota, and thereby alter signals of TEX_{86} in sediments. Besides temperature, pH can also alter compositions of brGDGTs (Weijers et al., 2007). Based on global soil data, the IIIa/IIa ratio shows a strong positive correlation with soil pH (R^2=0.51; Fig. 6). In our study, the majority of soils are acidic or neutral (pH<7.3) and only 8% of soil samples mainly from semi-arid and arid regions have pH of >8.0 (e.g., Yang et al., 2014a). In contrast, seawater is constantly alkaline with a mean pH of 8.2. With this systematic difference, bacteria living in soils tend to produce higher proportions of brGDGT IIa, whereas unknown marine organisms tend to biosynthesize higher proportions of brGDGT IIIa if they response to ambient pH in a similar way as soil

bacteria in term of biosynthesis of brGDGTs. It should be pointed out that unlike fairly stable pH of overlying sea water, the pH of pore waters in marine sediments can vary significantly, which may influence compositions of brGDGTs. Nevertheless, at current stage, the occurrence of higher IIIa/IIa values in marine sediments is most likely attributed to relatively higher pH and lower deep water temperature. Further studies are needed to disentangle relative importance of these two factors.

3.3 Implication of IIIa/IIa on other brGDGT proxies

Because brGDGTs can be produced in marine settings, they are no longer specific for soil organic matter, which inevitably affects brGDGT-derived proxies (e.g., BIT, MBT/CBT). The plot of BIT vs. IIIa/IIa on basis of global dataset shows that the IIIa/IIa ratio has the value of <0.59 for 90% of soil samples and >0.92 for 90% of marine sediments (Fig. 7). Considering this fact, we propose that the IIIa/IIa ratio of <0.59 and >0.92 represents terrestrial (or soil) and marine endmembers, respectively. The BIT index has the value of >0.67 for 90% of soils and <0.16 for 90% of marine sediments (Fig. 7). Overall, the BIT index decreased with increasing IIIa/IIa values($\mathrm{BIT} = 1.08 \times 0.28^{\frac{\mathrm{IIIa}}{\mathrm{IIa}}} - 0.03; \mathrm{R}^2 = 0.77$; Fig. 7), suggesting that both the IIIa/IIa and BIT are useful indexes for assessing soil organic carbon in marine settings. However, when the BIT index has an intermediate value (i.e., 0.16 to 0.67), it is not valid to determine the provenance of brGDGTs. For example, several marine samples having BIT values of ~0.35 show a large range of IIIa/IIa (0.4 to 2.4; Fig. 7), suggesting that the source of brGDGTs can vary case by case. Under this situation, the calculation of the IIIa/IIa ratio is strongly recommended.

The different IIIa/IIa values between land and marine endmembers may supply an approach to quantify the contribution of soil organic carbon in marine sediments. Similar to the BIT index, we used a binary mixing model to calculate percentage of soil organic carbon ($\%\mathrm{OC}_{\mathrm{soil}}$) as follow:

$$\%\mathrm{OC}_{\mathrm{soil}} = \left[\frac{[\mathrm{IIIa/IIa}]_{\mathrm{sample}} - [\mathrm{IIIa/IIa}]_{\mathrm{marine}}}{[\mathrm{IIIa/IIa}]_{\mathrm{soil}} - [\mathrm{IIIa/IIa}]_{\mathrm{marine}}}\right] * 100 \qquad (6)$$

Where $[IIIa/IIa]_{sample}$, $[IIIa/IIa]_{soil}$ and $[IIIa/IIa]_{marine}$ are the abundance ratio of brGDGT IIIa/IIa for samples, soils and marine sediments devoid of terrestrial influences, respectively.

We applied this binary mixing model to the East Siberian Arctic Shelf because the data of BIT, $\delta^{13}C_{org}$ and distance from river mouth are all available (Sparkes et al., 2015). With the distance from river mouth increasing from 25 to >700 km, the BIT, IIIa/IIa and $\delta^{13}C_{org}$ change from 0.95 to 0, 0.53 to 2.21 and –27.4‰ to –21.2‰, respectively, reflecting spatial variability of sedimentary organic carbon sources. For the BIT index, we used 0.97 and 0.01 as terrestrial and marine endmember values based on previous studies for Arctic surrounding regions (De Jonge et al., 2014; Peterse et al., 2014), which are similar to global average values (Hopmans et al., 2004). For $\delta^{13}C_{org}$, we chose –27‰ and –20‰ as C3 terrestrial and marine organic carbon endmembers (Meyers, 1997). For the IIIa/IIa ratio, we used a global average value of marine sediments (1.6) and soils (0.24), respectively, based on this study. By applying these endmember values into Eq. 6, we calculated percentage of soil organic carbon ($\%OC_{soil}$). We removed a few data points if their calculated $\%OC_{soil}$ were greater than 100% or below 0%. It should be noted that the endmember value will affect quantitative results, but does not change a general trend of $\%OC_{soil}$. The results based on all three parameters show a decreasing trend seawards (Fig. 8). However, the $\%OC_{soil}$ based on $\delta^{13}C_{org}$ is the highest (75±18%), followed by that from the IIIa/IIa ratio (58±15%) and then that from the BIT index (43±27%). This difference have been explained by that $\delta^{13}C_{org}$ is a bulk proxy for marine vs. terrestrial influence of sedimentary organic carbon (SOC), whereas the BIT index is for a portion of the bulk SOC, i.e., soil OC (Walsh et al., 2008) or fluvial OC (Sparkes et al., 2015). For the estimated $\%OC_{soil}$, $\delta^{13}C_{org}$ presents a stronger positive correlation with the IIIa/IIa ratio (R^2=0.49) than the BIT index (R^2=0.45), suggesting that the IIIa/IIa ratio may serve a better proxy for quantifying soil organic carbon than the BIT index because it is less affected by selective degradation of branched vs. isoprenoid GDGTs and high production of crenarchaea in marine environments (Smith et al., 2012).

382 4 Conclusions

383 The brGDGTs' distributions in three Bohai Sea cores and globally distributed soils
384 and marine sediments show that the brGDGTs IIIa/IIa ratio is lower than 0.59 in 90%
385 of soils, but higher than 0.92 in 90% of marine sediments, supporting that the IIIa/IIa
386 is a sensitive proxy for assessing soil vs. marine derived brGDGTs at regional and
387 global scales. The in situ production of brGDGTs in marine environments is a
388 ubiquitous phenomenon, which is particularly important for those marine sediments
389 with low BIT index (<0.16) where brGDGTs are exclusively of a marine origin. A
390 systemic difference of the IIIa/IIa value between soils and marine sediments reflects an
391 influence of pH rather than temperature on the biosynthesis of brGDGTs by source
392 organisms. Given these facts, we recommend to calculate the IIIa/IIa ratio before
393 estimating organic carbon source, paleo-soil pH and MAT based on the BIT and
394 MBT/CBT proxies. We also note a relatively large scatter of the IIIa/IIa ratio within
395 terrestrial and marine realms, and recently reported different environmental responses
396 of 5-methyl vs. 6-methyl brGDGTs. As a result, the separation of these two types of
397 isomers is needed in future studies in order to develop more accurate brGDGTs-based
398 proxies.

399

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405

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682 Fig.1. Chemical structures of branched GDGTs and crenarchaeol.

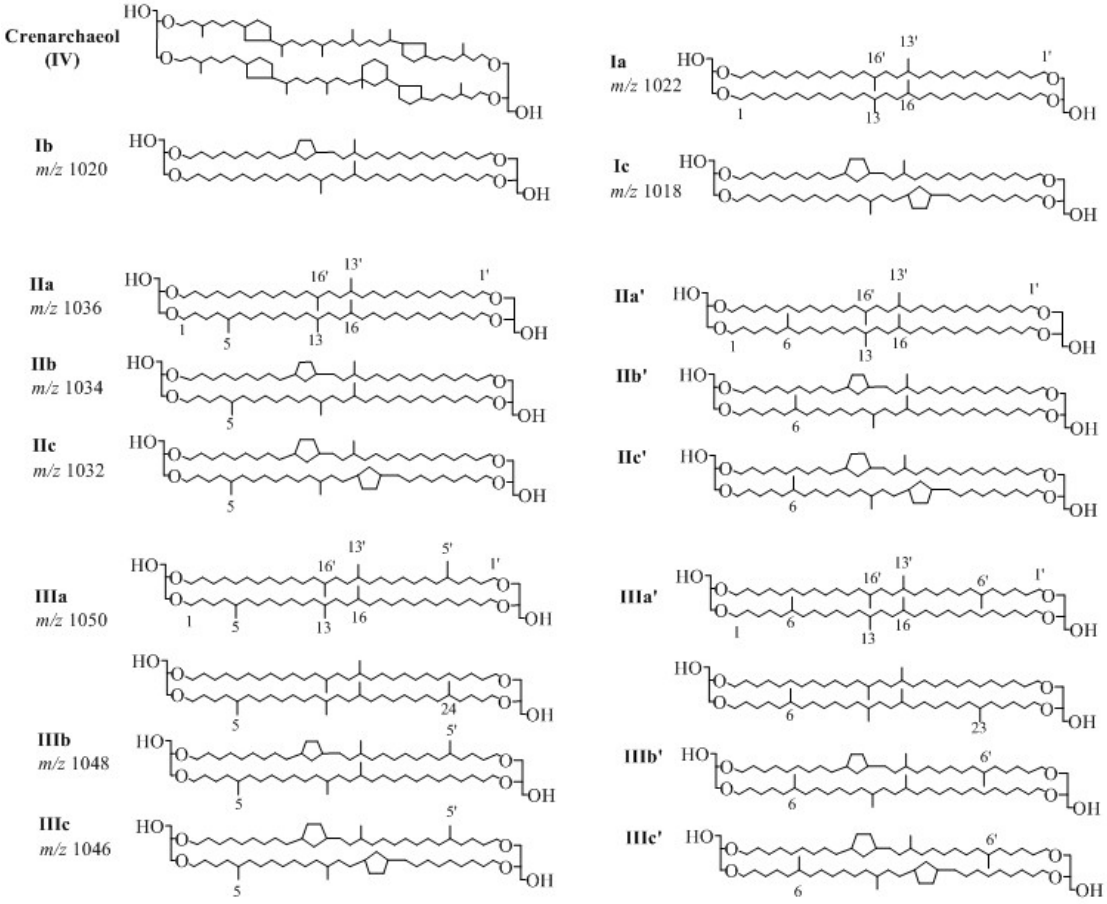

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Fig.2. Location of the samples used in this study. White circles and black circles indicate the soils and marine sediments, respectively. Red crosses denote three sediment cores (M1, M3 and M7) in the Bohai Sea. YR is the Yellow River.

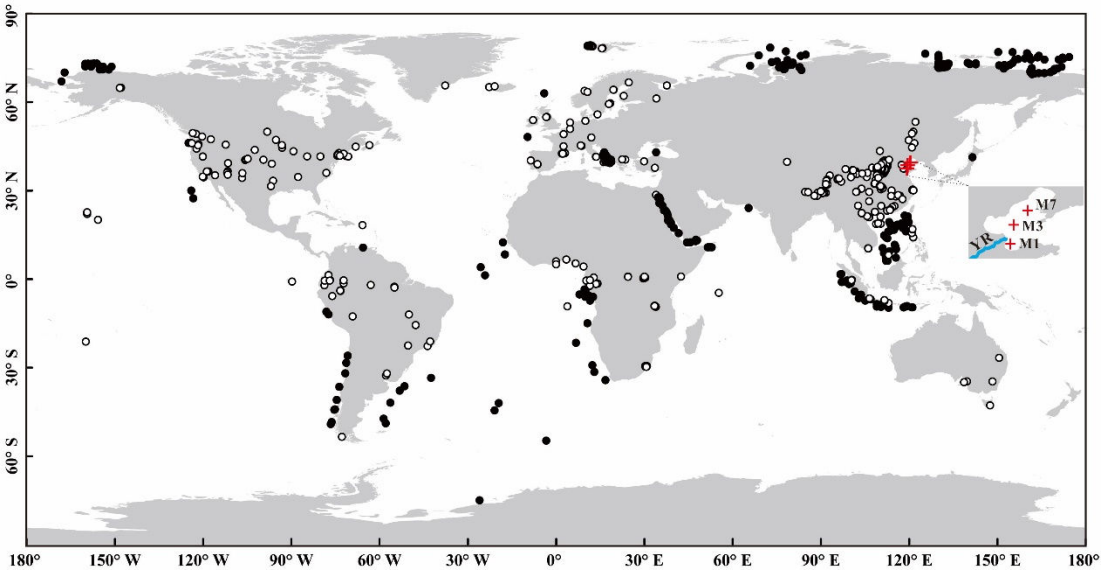

Fig.3. Averaged percentages of individual brGDGTs in soils (a), core M1 (b), M3 (c) and M7 (d). The soil data are from Yang et al. (2014a).

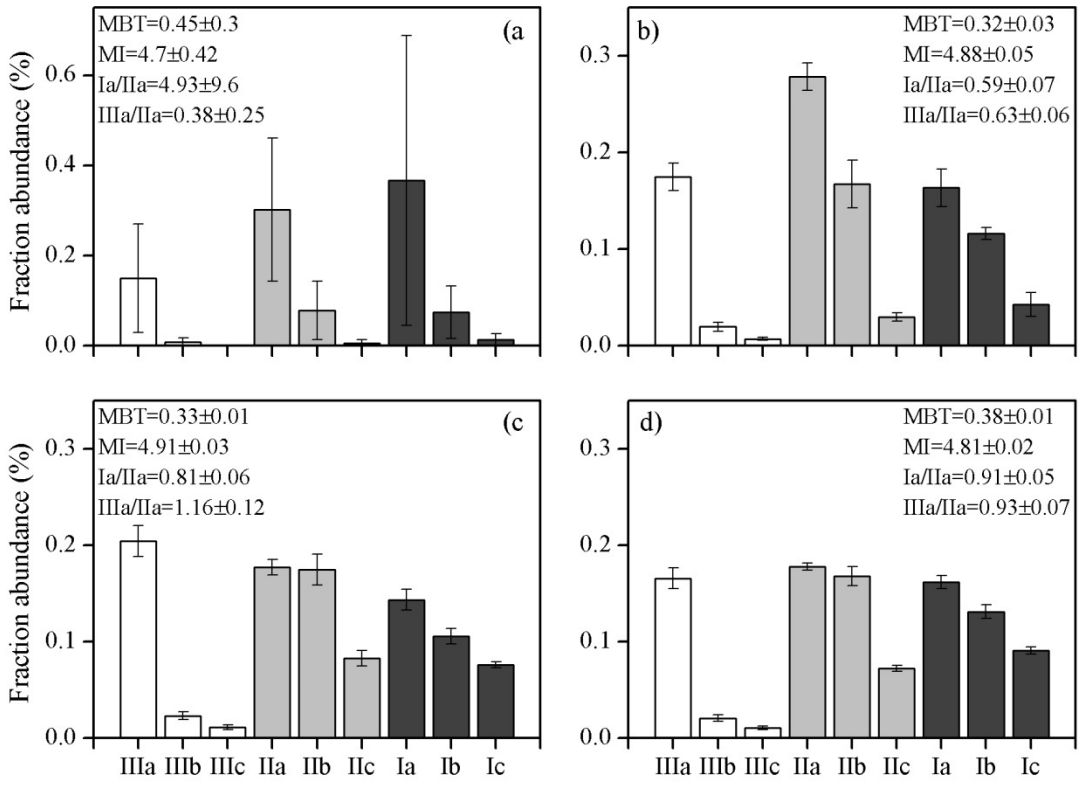

Fig. 4. a) The relationship between brGDGT IIIa/IIa ratio and the BIT index of samples from Peterse et al. (2009a); b) histograms of brGDGT IIIa/IIa ratio of the core lipids (CLs) and intact polar lipids (IPLs) in samples from De Jonge et al. (2015); c) the relationship between brGDGT IIIa/IIa ratio and the BIT index in samples from Sparkes et al. (2015); d) the relationship between brGDGT IIIa/IIa ratio and distance from river mouth in samples from Sparkes et al. (2015).

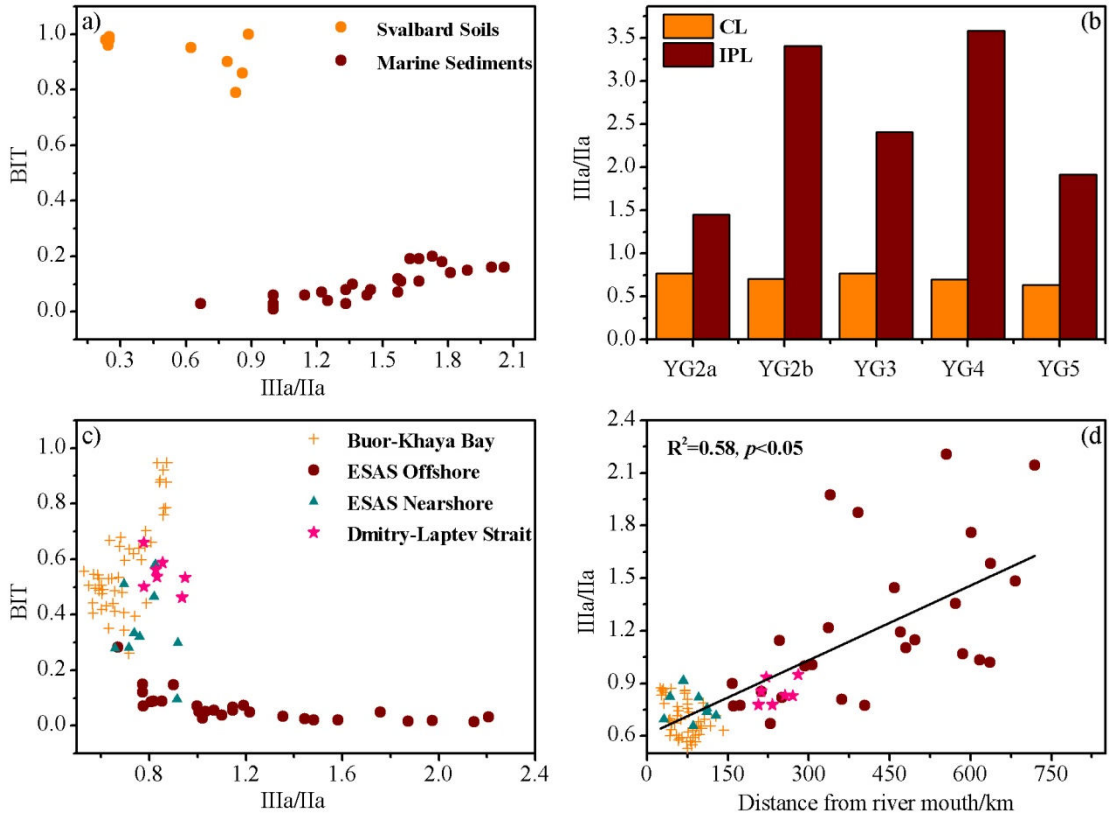

Fig. 5. Global distribution pattern of brGDGT IIIa/IIa ratio in soils and marine sediments.

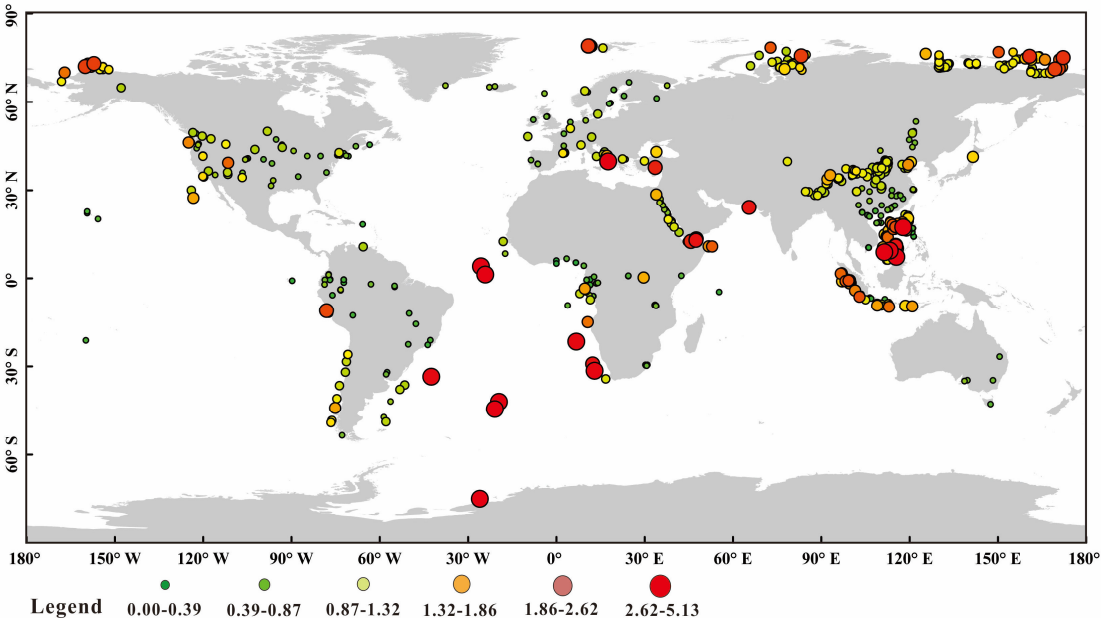

Fig. 6. A plot showing a positive correlation between soil pH and IIIa/IIa. The data are from Peterse et al. (2012) and this study.

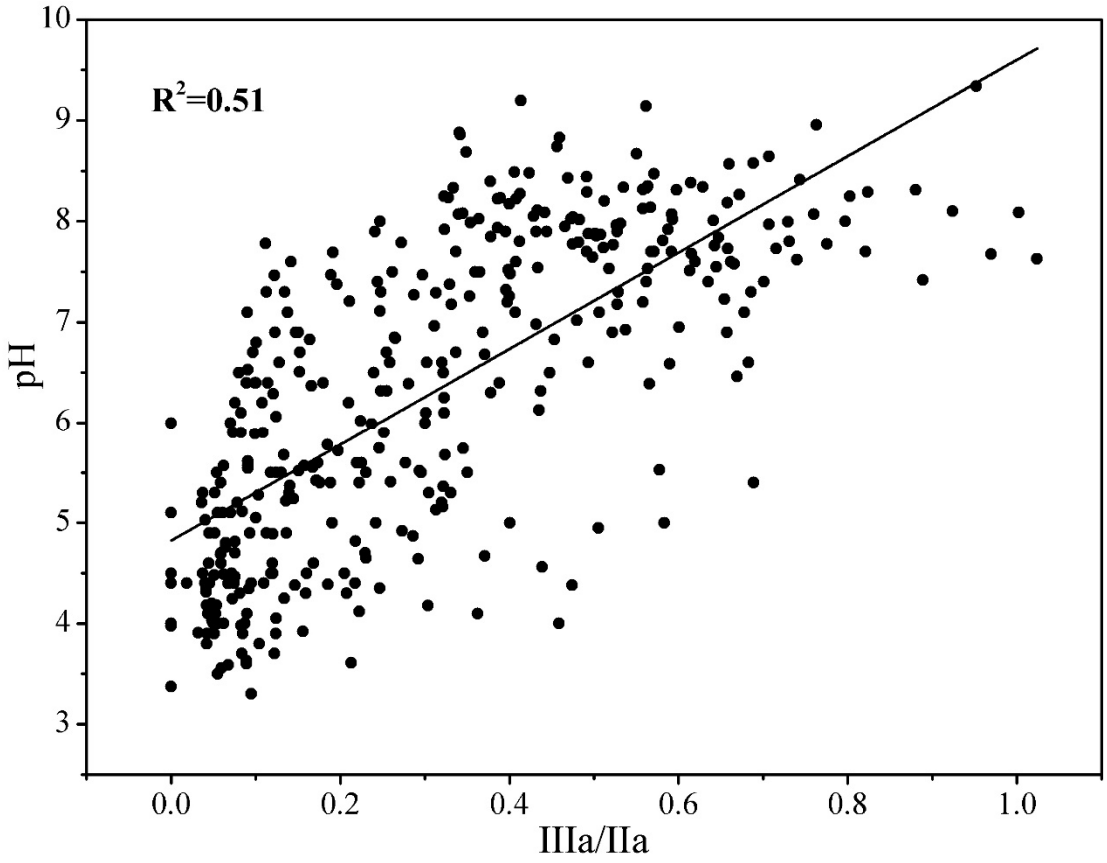

Fig. 7. Relationship between the IIIa/IIa ratio and the BIT index of globally distributed samples: soils (orange circle) and marine sediments (red circle). Dashed lines represent lower or upper threshold values for 90% of soils/sediments.

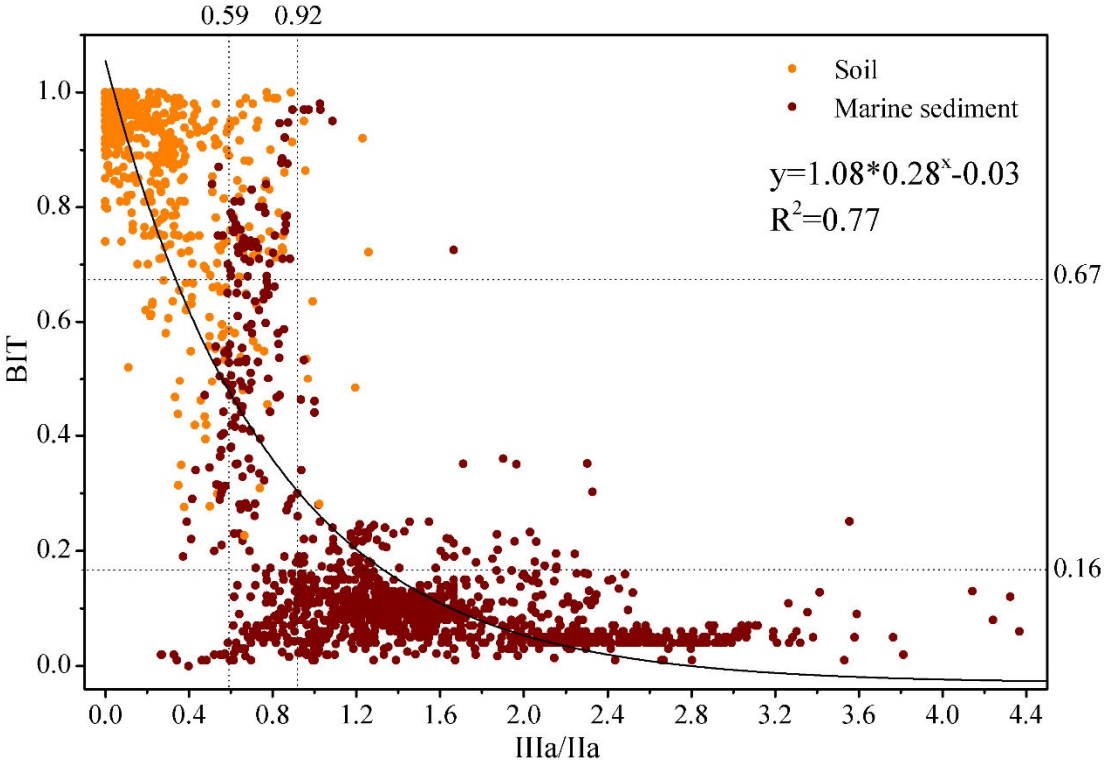

Fig. 8. Percentage of soil organic carbon ($\%OC_{soil}$) or terrestrial organic carbon ($\%OC_{terr}$) based on a binary mixing model of BIT (a), $\delta^{13}C_{org}$ (b) and IIIa/IIa (c) for the East Siberian Arctic Shelf (Sparkes et al., 2015).

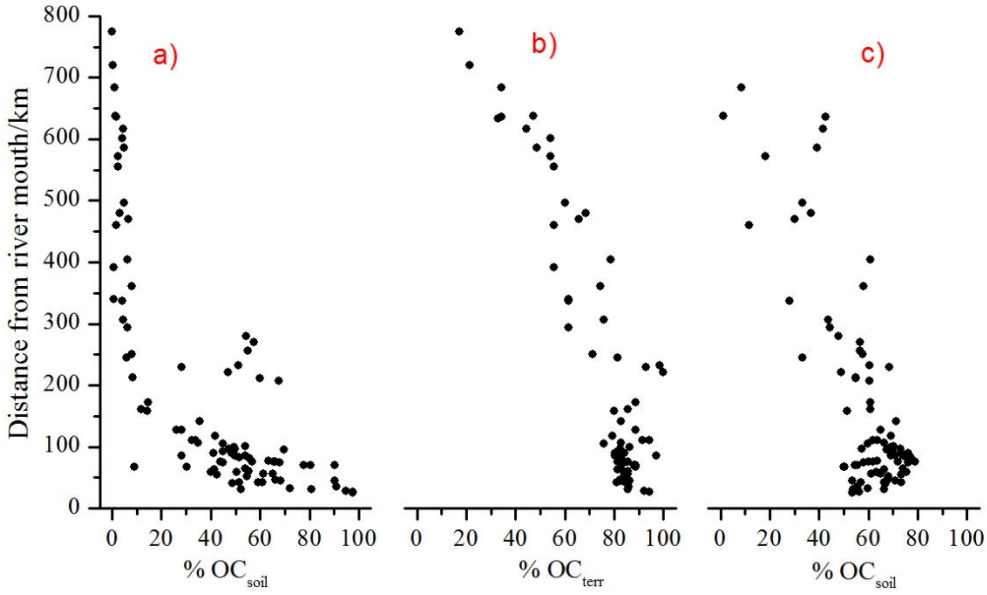

Table 1: Parameters including brGDGTs IIIa/IIa, Ia/IIa, the BIT index, MBT, MI, DC, percentages of tetra-, penta- and hexa-methylated brGDGTs, and the weighted average number of cyclopentane moieties (#rings for tetramethylated brGDGTs) based on the GDGTs from three cores (M1, M3 and M7; see figure 2) in the Bohai Sea. Different letters in parentheses (a, b, c, d) represent significant difference at the level of $p<0.05$.

Indexes	Soil	M1	M3	M7
IIIa/IIa	0.39±0.25 (a)	0.63±0.06 (b)	1.16±0.12 (c)	0.93±0.07 (d)
Ia/IIa	4.93±9.60 (a)	0.59±0.07 (b)	0.81±0.06 (b)	0.91±0.05 (b)
BIT	0.75±0.22 (a)	0.50±0.19 (b)	0.14±0.06 (c)	0.11±0.03 (c)
MBT	0.45±0.30 (a)	0.32±0.03 (b)	0.33±0.01 (b)	0.38±0.01 (ab)
MI	4.70±0.42 (a)	4.88±0.05 (b)	4.91±0.03 (b)	4.81±0.02 (ab)
DC	0.31±0.21 (a)	0.62±0.03 (b)	0.79±0.03 (c)	0.82±0.02 (c)
%tetra	0.45±0.30 (a)	0.32±0.03 (b)	0.33±0.01 (c)	0.38±0.01 (c)
%hexa	0.16±0.12 (a)	0.20±0.02 (b)	0.24±0.02 (b)	0.20±0.01 (b)
%penta	0.39±0.20 (a)	0.48±0.02 (b)	0.44±0.02 (b)	0.42±0.01 (b)
$\#Rings_{tera}$	0.20±0.15 (a)	0.39±0.03 (b)	0.47±0.02 (c)	0.47±0.02 (c)

Response to reviewer 1's comments:

On behalf of my coauthors, I really appreciate the reviewer to acknowledge the merit of our work. As the reviewer said, "The authors present an interesting manuscript, based on an extensive dataset compiled from previous publications. The figures are well chosen and convey the message clearly." In addition, we also thank the reviewer providing a number of useful comments, which are helpful to improve our manuscript.

Here, we tried our best to address the reviewer's comments point by point.

Anonymous Referee #1

1) Main comments (short, they are elaborated on below).

It is interesting that the IIIa/IIa ratio increase significantly in offshore marine sediments. However, the authors have not attempted to explain this by comparing the compounds this ratio is composed of (IIIa, IIIa', IIa or IIa'). This lessens the value of this study, by narrowing its implication for palaeoenvironmental studies. In the Kara Sea (Arctic Ocean), De Jonge et al. (2016) have clearly shown that brGDGT IIIa' increases in increasingly marine conditions (Yenisei River outflow), while brGDGT IIa' does not. The Iberian Sea (Sinninghe Damste et al., shows a different pattern). If the authors can shed light on which mechanism acts on marine sediments globally, this has implications for which temperature proxies can be used (also see De Jonge et al. (2016)).

Response: In our study, we used 2D LC-MS to separate 5-methy and 6-methyl brGDGTs. The reason we combined them together in the manuscript is that most previous studies using one dimensional LC-MS did not separate these two types of isomers. The first study to report 6-methyl brGDGTs was published in 2013 by De Jonge et al. So far only very limited studies paid attention to this issue. Nevertheless, we agree with the reviewer that the separation of 5-methyl and 6-methyl brGDGTs may provide more accurate proxies for source and environmental information of brGDGTs. In the revised manuscript, we added comment on this points We wrote in the conclusion as "We also note a relatively large scatter of the IIIa/IIa ratio within both terrestrial and marine realms, and different environmental responses of 5-methyl and 6-methyl brGDGTs (e.g., De Jonge et al., 2014, 2016; Xiao et al., 2015). As a result, the separation of these two types of isomers is needed in future studies to develop more accurate brGDGTs' proxies." We expect more data about 5-methyl and 6-methyl brGDGTs available in future, so we can compile them and develop new molecular proxies.

2) I find the reasoning behind the absence of a temperature difference between soil/peat and marine brGDGTs incomplete. I expect a very large difference in temperature between soil and marine bottom water, even at similar latitudes.

Response: This is a good comment. Recent studies suggested that the production of GDGTs in deep water is possible. If so, large temperature gradient between surface and deep water in ocean inevitably affects brGDGTs' compositions. In the revised manuscript, we consider this factor for different brGDGTs' compositions between land and sea. In section 3.2, we rewrote the whole paragraph (line 486-549) as "Why do marine sediments have higher IIIa/IIa values than soils? It has been reported that relative number of methyl groups positively correlates with soil pH and negatively correlates with MAT (Peterse et al., 2012; Weijers et al., 2007b). The IIIa/IIa ratio is actually an abundance ratio of hexamethylated to pentamethylated brGDGT, and thus is also affected by ambient temperature and pH. Unlike iGDGTs which is well known to be mainly produced by Thaumarchaeota (Schouten et al., 2008; Sinninghe Damsté et al., 2002), the marine source of brGDGTs remains elusive. Here, we assume that marine organisms producing brGDGTs response to ambient temperature in the same way as those soil bacteria producing brGDGTs, i.e., a negative correlation between relative number of methyl group of brGDGTs and ambient temperature. In order to evaluate temperature effect on brGDGTs' compositions, we need consider the locale where brGDGTs are produced. If brGDGTs in marine environments are predominantly produced in euphotic zone, a significant difference for the IIIa/IIa ratio would not be observed between soils and marine sediments because both soils and marine sediments are globally distributed, leading to no systematic difference between soil temperature and sea surface temperature. Alternatively, if brGDGTs in marine sediments are partially derived from deep-water dwelling or benthic organisms, cold deep water (generally 1–2 ºC) would cause higher IIIa/IIa values in marine sediments, as we observed. Besides temperature, pH can also alter compositions of brGDGTs (Weijers et al., 2007). Based on global soil data, the IIIa/IIa ratio shows a strong positive correlation with soil pH (R^2=0.51; Fig. 6). In our study, the majority of soils are acidic or neutral (pH<7.3) and only 8% of soil samples mainly from semi-arid and arid regions have pH of >8.0 (e.g., Yang et al., 2014a). In contrast, seawater is constantly alkaline with a mean pH of 8.2. With this systematic difference, bacteria living in soils tend to produce higher proportions of brGDGT IIa, whereas unknown marine organisms tend to biosynthesize higher proportions

of brGDGT IIIa if they response to ambient pH in a similar way as soil bacteria in term of biosynthesis of brGDGTs. Taking together, we attributed the occurrence of higher IIIa/IIa values in marine sediments to higher pH and lower deep water temperature. Further studies are great needed to disentangle relative importance of these two factors."

3) The introduction of previous studies describing marine in-situ produced brGDGTs is too concise. Furthermore, in the discussion I miss how the conclusions from the authors fit with previously published manuscripts? Can we say anything about the water depths at which brGDGTs are produced?

Response: we discussed in more details about in-situ of brGDGTs in the revised manuscript. Please see our response below (line 97-90). To the best of our knowledge, there is no study addressing production of brGDGTs at different water depth. However, a recent study from Kim et al. (2015) has demonstrated an influence of deep water derived iGDGTs on TEX86. So if brGDGTs are also produced in deep water, it would alter brGDGTs' proxies. We discussed this point in section 3.2 "Why do marine sediments have higher IIIa/IIa values than soils?". From line 535 to 543, we said "Alternatively, if brGDGTs in marine sediments are partially derived from deep-water dwelling or benthic organisms, cold deep water (generally 1–2 ºC) would cause higher IIIa/IIa values in marine sediments, as we observed in this study. Although to the best of our knowledge, there is no study reporting in situ production of brGDGTs throughout water column in ocean. Recent studies (Kim et al., 2015; Taylor et al., 2013) have suggested that Thaumarchaeota thriving in the deeper, bathypelagic water-column (>1000 m water depth) biosynthesized iGDGTs with different compositions as surface dwelling Thaumarchaeota, and thereby influences signals of TEX_{86}."

Minor comments:

4） L14. Rephrase this so the "presumed source" of brGDGTs (soil, peat) is introduced first.

Response: we made change in the revised manuscript as: "Presumed source specificity of branched glycerol dialkyl glycerol tetraethers (brGDGTs) from bacteria thriving in soil/peat and isoprenoid GDGTs (iGDGTs) from aquatic organisms led to the development of several biomarker proxies for biogeochemical cycle and paleoenvironment."

5） L33: Use 'have been' instead of 'have become'.

Response: we made correction according to reviewer's comment.

6） L43: Rephrase, this is a confusing sentence. The stereoconfiguration of the glycerol moiety indicates that they are produced by bacteria, not the fact that they are abundant in soils.

Response: We rewrote as "In contrast, the 1,2-di-O-alkyl-sn-glycerol configuration of brGDGTs is interpreted as an evidence for a bacterial rather than archaeal origin for brGDGTs (Sinninghe Damsté et al., 2000; Weijers et al., 2006)" in the revised manuscript.

7) L49: Please include that 15 brGDGT compounds are generally encountered in soils. Besides the variation in the number of methyl groups and cyclopentane moieties, the location of the outer branches has been shown to shift as well.

Response: we accept this suggestion, and rewrote sentences as "So far, only two species of Acidobacteria were identified to contain one brGDGT with two 13,16-dimethyl octacosanyl moieties (Sinninghe Damsté et al., 2011), which is contrast to high diversity and ubiquitous occurrence of 15 brGDGT isomers in environments (De Jonge et al., 2014; Weijers et al., 2007b)."

8) L 54: Use Thaumarchaeota instead of crenarchaea.

Response: we made change in the revised manuscript.

9) L 58: Here, you can also refer to 'Weijers et al. (2014), Constraints on the sources of branched tetraether membrane lipids in distal marine sediments, OG 72'.

Response: We added the reference of "Weijers et al., 2014" in the revised manuscript.

10) L 87-90. As this manuscript discusses brGDGTs produced in marine systems, I would expand a bit more on all studies that have provided evidence for the in-situ production of brGDGTs in the marine environment (instead of just listing them up). Now, only the recent Sinninghe Damste (2016) paper is introduced.

Response: We added more discussion about potential marine-derived brGDGTs. We wrote as "Peterse et al. (2009) compared the brGDGT distribution in Svalbard soils and nearby fjord sediments, and found that concentrations of brGDGTs (0.01–0.20 μg/g dw) in fjord sediments increased towards the open ocean and the distribution was strikingly different from that in soil. Zhu et al. (2011) examined distributions of GDGTs in surface sediments across a Yangtze River-dominated continental margin,

and found evidence for production of brGDGTs in the oxic East China Sea shelf water column and the anoxic sediments/waters of the Lower Yangtze River. At the global scale, Fietz et al. (2012) reported a significant correlation between concentrations of brGDGTs and crenarchaeol ($p < 0.01$; $R^2 = 0.57–0.99$), suggesting that a common or mixed source for brGDGTs and iGDGTs are actually commonplace in lacustrine and marine settings. More recently, Sinninghe Damsté (2016) reported tetraethers in surface sediments from 43 stations in the Berau River delta (Kalimantan, Indonesia), and this result, combined with data from other shelf systems, supported a widespread biosynthesis of brGDGTs in shelf sediments especially at water depth of 50–300 m. ”

11) L 91-99 could be restructured, they are not easy to understand.

Response: We rewrote this paragraph as “However, so far no robust molecular indicator is available for estimating source of brGDGTs in marine environments. Considering this, we conduct a detailed study about GDGTs in three cores from the Bohai Sea which are subject to the Yellow River influence to different degree. Our purpose is to evaluate the source discerning capability of different brGDGT parameters, from which the most sensitive parameter is selected and applied for globally distributed marine sediments and soils to test whether it is valid at the global scale. Our study supplies an important step for improving accuracy of brGDGT-derived proxies and better understanding the marine carbon cycle and paleoenvironments.”

12) L 106: ‘the marine carbon cycle’

Response: we made change in revised manuscript.

13) L 112. ‘the mean depth is’ and ‘the Bohai Strait, at the east’.

Response: we made the change according to reviewer’s suggestion.

14) L 114: ‘the second largest river in the world, concerning sediment load (+reference)’

Response: We rewrote this sentence and added the reference as “Several rivers, including Yellow River, the second largest river in the world in terms of sediment load (Milliman and Meade, 1983), drain into the Bohai Sea with a total annual runoff of 890×10^8 m^3.”

15) L 115: ‘One gravity core of 64 cm was: : :’

Response: we made correction in the revised manuscript.

16) L 118: respectively can be removed here.

Response: we already removed "respectively".

17) L 125: If this extraction and separation protocol has been described elsewhere, you can simply refer to this original publication. The same goes for the analysis of the GDGTs on the LC system.

Response: This is a good suggestion. In the revised manuscript, we delete the details about extraction and analysis methods. We started this paragraph as "The detailed procedures for lipid extraction and GDGT analyses were described in previous studies (Ding et al., 2015; Xiao et al., 2015)."

18) L 183: This can be rewritten as: De Jonge et al., 2015, 2016.

Response: We made change.

19) L 206: Can the authors not give an indication at which BIT values (both on the local and global scale) the proportion of marine brGDGT becomes problematic? This would be useful from the viewpoint of palaeoclimate reconstructions.

Response: As we stated in the manuscript, "However, the BIT index itself has no ability to distinguish terrestrial vs. aquatic brGDGTs because brGDGTs and crenarchaea used in this index are thought to be specific for soil organic carbon and marine organic carbon, respectively (Hopmans et al., 2004)". Only the combination of BIT and IIIa/IIa can reveal that when BIT is lower than 0.16, a contribution of marine brGDGTs becomes problematic, which was discussed in section 3.3 and figure 7.

20) L 235-237: Is a repetition of the L 237-242.

Response: we deleted this sentences in the revised manuscript.

21) L282. It surprises me that the authors indicate here that Crenarchaeota/Thaumarchaeota are the probable producers of marine brGDGTs. Is there any indication that this would be the case? Alternatively, I would remove this statement.

Response: We agree with reviewer that more solid evidence is needed to draw such conclusion. Trommer et al. (2009) indeed postulated the existence of distinct crenarchaeota community in the Red Sea due to unusally environmental condition. Considering these facts, we did not specify crenarchaeota in the revised manucript. In stead we rewrote the sentecne as "Under such extreme environment, distinct microbial populations may be

developed and produced GDGTs different from that in other marine settings (Trommer et al., 2009)".

22) L303. The argument that continental and marine temperatures are significantly different is put aside much too quickly. Indeed, they are both globally distributed, but the temperature of your water bodies will be much more stable throughout the year (which has an implication of the production temp as soil-derived brGDGTs are thought to be produced mainly in spring-autumn, especially at sites that are partially frozen throughout the year. Furthermore, if marine brGDGTs are produced at the sediment/water interface, this will of course be much colder than the sea surface temperature. Taking this into account, I doubt that the authors will be able to make a strong case on their proposed absence of a temperature difference between soil and marine brGDGTs.

Response: This is a good comment and already mentioned in major comment. We added detailed discussion in our revised manuscript. Please see our response for Comment #2, particularly about production of GDGTs in surface and deep water with large different temperature.

23) L304. If the authors want to discuss this trend between soil pH and III/II, they have to provide a plot. Does this trend also apply for more extreme pH values? Can it be strengthened by determining which compound causes this trend (IIa, IIa', IIIa, IIIa')?

Response: Good suggestion. We added a figure to show a trend between soil pH and IIIa/IIa (Fig. 6). We agree the separation of 5-methyl and 6-methyl brGDGTs by 2D HPLC-MS may strengthen our hypothesis. However, most available data on brGDGTs did not distinguish these two types of isomers. So we still combine 5-methyl and 6-methyl brGDGTs in current study. But we, along with several groups, are currently using advanced HPLC-MS method to quantify 5-methyl and 6-methyl brGDGTs for more samples. We plan to review 5-methyl and 6-methyl brGDGTs in future when sufficient data are available, but at current stage, this is beyond the scope of this manuscript.

24) L308: The pH of marine water is indeed fairly stable, but it can be very different in pore waters in the sediments. This should be mentioned.

Response: The production of brGDGTs in pore water of sediments cannot be excluded, although they are likely not as important as water column. In the revised manuscript, we added the discussion as "It should be pointed out that unlike fairly stable pH of overlying sea water, the pH of pore waters in marine sediments can vary significantly, which may influence compositions of brGDGTs. Nevertheless, at current stage, the occurrence of

higher IIIa/IIa values in marine sediments is most likely attributed to relatively higher pH and lower deep water temperature. Further studies are needed to disentangle relative importance of these two factors."

25) L367: 'and a compilation of'

Response: we added "a" before compilation.

26) L364-367: I do not agree that the authors have enough evidence and data on this to make this conclusion.

Response: we agree more studies are needed to unambiguously determine source of brGDGTs in marine environments. So in the end of the revised manuscript, we added sentences as "We also note a relatively large scatter of the IIIa/IIa ratio within both terrestrial and marine realms, and different environmental responses of 5-methyl and 6-methyl brGDGTs (e.g., De Jonge et al., 2014, 2016; Xiao et al., 2015). As a result, the separation of these two types of isomers is needed in future studies to develop more accurate brGDGTs' proxies."

27) References: please check the manuscripts guidelines. Journal names are to be abbreviated.

Response: we update the references with abbreviation journal name.

28) General: In the manuscript text, the authors should pay attention to the order of references. Older references should come first.

Response: we reorganized our references according to the requirement of Biogeosciences.

On behalf of my coauthor, I really appreciate the reviewer 2 supplying very detailed comments, which are helpful to improve our manuscript. Overall, the reviewer 2 acknowledge the merit of our paper. As he or she said that "the work by Xiao et al. is a very interesting and valuable contribution to the study of the distribution and origin of branched GDGTs (brGDGT) in mesophilic marine environments and their use as climate proxies" and "This finding, in my opinion, should grant the paper publication in a number of (bio)geochemical journals".

The main concern of the reviewer 2 is not 100% sure of in situ brGDGTs in aquatic environments. In our opinion, more and more studies support that brGDGTs are also biosynthesized by aquatic organisms. Presently, the in-situ production of brGDGTs in aquatic environments has been accepted by most organic geochemists. For examples, Peterse et al. (2009a), Zhu et al. (2011), Liu et al. (2014) Weijers et al. (2014) and Zell et al. (2014) all observed different brGDGT compositions between marine sediments from different seas and soils on adjacent lands, so they proposed in situ production of brGDGTs in marine environments. Similar conclusions have been reached for lacustrine settings by Sinninghe Damsté et al. (2009), Tierney & Russell (2009) and Tierney et al. (2012) as well as for rivers by Zhu et al. (2011), De Jonge et al. (2015), French et al. (2015) and Zell et al., (2015). Most those studies were published after 2010, so more and more organic geochemists accepted the view that brGDGTs were produced in aquatic environments.

Peterse et al. (2009) compared the brGDGT distribution in Svalbard soils and nearby fjord sediments, and found that concentrations of brGDGTs (0.01–0.20 μg/g dw) in fjord sediments increased towards the open ocean and the distribution was strikingly different from that in soil. Zhu et al. (2011) examined distributions of GDGTs in surface sediments across a Yangtze River-dominated continental margin, and found evidence for production of brGDGTs in the oxic East China Sea shelf water column and the anoxic sediments/waters of the Lower Yangtze River. At the global scale, Fietz et al. (2012) reported a significant correlation between concentrations of brGDGTs and crenarchaeol ($p < 0.01$; $R2 = 0.57–0.99$), suggesting that a common or mixed source for brGDGTs and iGDGTs are actually commonplace in lacustrine and marine settings. Weijers et al. (2014) found that distributions of African dust-derived brGDGTs were similar to those of soils but different from those of distal marine sediments, providing a possibility to distinguish terrestrial vs. marine brGDGTs based on molecular compositions. More recently, Sinninghe Damsté (2016) reported tetraethers in surface sediments from 43 stations in the Berau River delta (Kalimantan, Indonesia), and this result, combined with data from other shelf systems, are coherent with the hypothesis that brGDGTs are in situ produced in shelf sediments especially at water depth of 50–300 m. In the remote ocean where no direct impact from land erosion via rivers takes place, eolian transport and in situ production are major contributors for brGDGTs.

So in our opinion, based on recent studies and our study, the in-situ production of brGDGTs is a ubiquitous phenomenon in marine environments, as our title states.

Comment: Lines 34 and 35: to estimate environmental variables in the past
Response: we accept this suggestion and rewrote the sentence as "These lipids have been a focus of attention of organic geochemists for more than ten years because they can be used to estimate environmental variables in the past such as temperature, soil pH, organic carbon source and microbial community structure"

Comment: Line 45: the attribution of the brGDGTs is still hypothetical. But in any case it is unclear. why the authors claim that their preferential occurrence in soils/peats means that they are derived from bacteria
Response: the source assignment of branched GDGTs to bacteria is based on structural configuration (1,2-di-*O*-alkyl-*sn*-glycerol configuration). It is generally accepted by Organic Geochemists.

Comment: Li 48-50: the text should be rewritten, it is unclear what the authors are trying to say
Response: in revised manuscript, we rewrote the sentences as: "So far, only one brGDGT with two 13,16-dimethyl octacosanyl moieties was unambiguously detected in two species of

Acidobacteria (Sinninghe Damsté et al., 2011), which hardly explains high diversity and ubiquitous occurrence of up to 15 brGDGT isomers in environments (Weijers et al., 2007b; De Jonge et al., 2014). Therefore, other biological sources of brGDGTs, although not yet identified, are likely."

Comment: Li 58-59: the BIT index is used for what?
Response: we rewrote the sentence as "Since its advent, the BIT index has been increasingly used to trace soil organic matter in different environments" in revised manuscript.

Comment: Li 58-60: in here the authors should also comment the often overlooked drawback of the BIT index, namely that is dependent on the input of chrenarcheol, which is linked to marine productivity. Consequently, BIT values are not just dependent on the inputs of soil brGDGTs, but also on the productivity of marine Archaea. For instance, sites with equal inputs of terrestrial brGDGTS but different local productivity would display different BIT values. There are a number of references out there discussing this issue, for instance:
*Herfort, L., S. Schouten, J. P. Boon,M. Woltering, M. Baas, J. W. H. Weijers, and J. S. Sinninghe Damsté (2006), Characterization of transport and deposition of terrestrial organic matter in the southern North Sea using the BIT index, Limnol. Oceanogr., 51, 2196–2205, doi:10.4319/lo.2006. 51.5.2196.
*Fietz, S., Martínez-Garcia, A., Huguet, C., Rueda, G., & Rosell-Melé, A. (2011). Constraints in the application of the Branched and Isoprenoid Tetraether index as a terrestrial input proxy. Journal of Geophysical Research, 116(C10), 1–9.
*Smith, R. W., Bianchi, T. S., & Savage, C. (2010). Comparison of lignin phenols and branched/isoprenoid tetraethers (BIT index) as indices of terrestrial organic matter in Doubtful Sound, Fiordland, New Zealand. Organic Geochemistry, 41(3), 281–290. http://doi.org/10.1016/j.orggeochem.2009.10.009
Response: This is a good comment. In the revised manuscript, we added this comment as well as related references. We wrote as "However, the BIT index is not just dependent on the abundance of brGDGTs, which reflects the input of soil organic matter, but also on the abundance of chrenarcheol, which is linked to marine productivity (e.g., Herfort et al., 2006； Smith et al, 2010; Fietz et al., 2011)."

Comment: Li 76: "The premise of all brGDGT", do you the authors mean: the underlying assumption?
Response: Yes, that is what we meant. In order to avoid confusion, we changed "premise" into "underlying assumption" in the revised manuscript.

Comment: Li 79: "supporting in situ production of brGDGTs": the authors cited hypothesized the occurrence of in situ production, so their studies supported the hypothesis of the occurrence of...
Response: Actually, there are a number of studies supporting in-situ production of brGDGTs aquatic environments such as lake, river and marginal seas, such as Svalbard, Norway(Peterse et al., 2009a, OG); Yangtze River and estuary (Zhu et al., 2011, OG); Black Sea and Cariaco Basin (Liu et al., 2014, Mar Chem); equatorial West African coast (Weijers et al., 2014, OG); Portuguese margin (Zell et al., 2014; Biogeosciences). So the in-situ production of brGDGTs is generally accepted by organic geochemists.

Comment: Li 89: instead of "supported" use "findings were coherent with the hypothesis that brGDGTs are in situ produced in marine environaments".
Response: Although we are pretty sure for the existence of in-situ production of brGDGTs in

aquatic environments (see our response above), we rewrote as “this result, combined with data from other shelf systems, are coherent with the hypothesis that brGDGTs are in situ produced in shelf sediments especially at water depth of 50–300 m.”

Comment: Li 91: instead of “river” use “fluvial inputs or run off”
Response: we accepted this comment and changed “river” into “fluvial inputs”

Comment: Li 93-94: brGDGTs have not been analyzed in that many dust samples to date, but it may be obvious to assume in the meantime that their concentration in dust will be as high, proportionally, to the contents of soil particles in dust. In this section it is relevant to cite as well the just published paper by Yamamoto et al., 2016, GCA, 191,15 October 2016, Pages 239–254.

Comment: Li 95: “became”: why just in the past?
Response: we change “became” into “are major contributors for brGDGTs”.

Comment: Li 112: mean depth
Response: we already corrected this spelling mistake.

Comment: Li 115-116: One 64 cm long gravity core
Response: we accepted this suggestion and made change in revised manuscript.

Comment: Li 117: namely?
Response: we deleted “namely” and rewrote the sentence as “while other two cores, M3 (38.66°N, 119.54°E; 53 cm long) and M7 (39.53°N, 120.46°E; 60 cm long), were collected in July 2013”.

Comment: Li 121-122: I would rephrase “cores cover the sedimentation period of less than 100 years”
Response: We rewrote the sentence as “showing that the age of the bottom sediments was less than 100 years old”.

Comment: Li 125: samples were ground with a mortar and pestle
Response: Based on this suggestion and comment from reviewer 1, we rewrote as “The detailed procedures for lipid extraction and GDGT analyses were described in previous studies (Ding et al., 2015; Xiao et al., 2015). Briefly, the homogenous freeze-dried samples were ultrasonically extracted with dichloromethane (DCM)/methanol (3:1 v:v).”

Comment: Li 137: Define “EtOAc”
Response: We deleted EtOAc in the revised manuscript, and rewrote the part of analytical procedure in revised manuscript.

Comment: Li 138; I would rephrase “Samples were injected: : :”, where?
Response: We rewrote the part of Lipid Extraction and Analyses according to the reviewers’ comments. Please see the revised manuscript for details (Section 2.2).

Comment: Li 139: As this is relatively novel, I would indicate from which reference(s) the HPLC method is derived.
Response: In the beginning of the paragraph, we added the references as “The detailed procedures for lipid extraction and GDGT analyses were described in previous studies (Ding et al., 2015; Xiao et al., 2015)”.

Comment: Li 175: “The dataset in this study are composed of GDGTs from..” absolute/relative concentrations?, fluxes?
Response: We changed into “The dataset in this study are composed of relative abundance of GDGTs and derived parameters from 1354 globally distributed soils and 589 marine sediments (Fig. 2 and supplementary data)” in the revised manuscript.

Comment: Li 177: I would rephrase “and have water depth”
Response: we changed into “and the water depth ranges from 1.0 to 5521 m”.

Comment: Li 197: I would rewrite “Both iGDGTs including crenarchaea and brGDGTs”. Chrenarchaea or chrenarcheaol?
Response: We rewrote as “A series of iGDGTs including crenarchaeol and brGDGTs including 5-methyl and 6-methyl isomers were detected in Bohai Sea sediments.” In the revised manuscript.

Comment: Li 206: “expectable”?
Response: we changed into “Such difference is not surprising”.

Comment: Li 207-210: iGDGTs are found in soils too.
Response: It is true iGDGTs are present in soils at low abundance. So we rewrote the sentence as “However, the BIT index itself has no ability to distinguish terrestrial vs. aquatic brGDGTs because brGDGTs and crenarchaeol used in this index are thought to be specific for soil organic carbon and marine organic carbon, respectively (Hopmans et al., 2004), although crenarchaeol is also present in soils at low abundance (Weijers et al., 2006).”

Comment: Li 208: the statement does not make much sense as the BIT was not “designed” for this purpose as it has already been discussed
Response: We agree on that BIT is designed for estimation of terrestrial organic matter in aquatic environments in Hopmans et al. (2004). However, more and more recent studies suggest that branched GDGTs are also in situ products in aquatic environments, such as lake, river and seas. Although the BIT index is still useful for two end-members (aquatic and soil organic matter), but it lack the capability to discern the source of branched GDGTs from terrestrial or in situ aquatic origin in seas. In contrast, the character of brGDGTs’ composition (excluding crenarchaeol) can be used to estimate the source of brGDGTs, such as IIIa/IIa ratio proposed in our study. Nevertheless, we rewrote the sentence here as “However, the BIT index itself has no ability to determine the source of brGDGTs (terrestrial vs. aquatic) because brGDGTs and crenarchaeol used in this index are thought to be specific for soil organic carbon and marine organic carbon, respectively (Hopmans et al., 2004), although crenarchaeol is also present in soils at low abundance (Weijers et al., 2006).”

Comment: Li 212-214: I would rephrase this section “all parameters except MI can distinguish Chinese soils from Bohai Sea sediments”
Response: we accept this suggestion and rewrote this section. In the revised manuscript, we rewrote as “We performed ANOVA for a variety of brGDGTs’ parameters. All results except from MI show a significant difference between Chinese soils and Bohai Sea sediments. The IIIa/IIa ratio is the most sensitive parameter which can completely separate the samples into four groups, Chinese soils (0.39±0.25; Mean±SD; same hereafter), M1 sediments (0.63±0.06), M3 sediments (1.16±0.12) and M7 sediments (0.93±0.07).”

Comment: Li 234-235: “enhanced IIIa/IIa values in the Bohai Sea sediments is caused by in 234 situ production of brGDGTs.” The statement should be rephrased to differentiate between

actual findings (i.e. IIIa/IIa values), and their proposed interpretation(s) (i.e. in situ production).
Response: It

Comment: Li 237-239: "The site M1 is adjacent to the Yellow River mouth and receives the largest amount of terrestrial organic matter, causing lower IIIa/IIa values". Again, the authors should rephrase the statement to indicate which is their interpretation of the IIA/IIa values, as they do not prove what causes the lower IIIa/IIa values. The same applies to text in lines 262, 272-273, 315, 371-374. Li 240: "comprises of the least amount of terrestrial organic matter", please justify this statement. Li 242: "strongly", why?, is this a subjective claim or is backed up by some stats.?
Response: Good comment. We rewrote the sentences as "In our study, the site M1 is adjacent to the Yellow River mouth and receives the largest amount of terrestrial organic matter, causing lower IIIa/IIa values (0.63±0.06). In contrast, the site M3 located in central Bohai Sea comprises of the least amount of terrestrial organic matter, resulting in higher IIIa/IIa values (1.16±0.12). The intermediate IIIa/IIa values at the site M7 (0.93±0.07) is attributed to moderate land erosion nearby northern Bohai Sea (Fig. 2). These GDGTs' results, consistent with other terrestrial biomarkers such as C29 and C31 n-alkanes and C29 sterol (data not showed here), suggest that the higher IIIa/IIa values in the Bohai Sea sediments compared to Chinese soils (0.39±0.25) is most likely caused by in situ production of brGDGTs."

Comment: Li 246-247: The authors should indicate that they try to validate the ratio as a proxy for something, not to validate the ratio itself, or are they also trying to assess if the IIa and IIIa are ubiquituous?
Response: We added the sentence to state this point. In the beginning of this paragraph, we wrote as "Similar to Bohai Sea in this study, the compounds brGDGT IIa and IIIa are also ubiquitously present in these environments."

Comment: Li 258: compiling or compilation?
Response: We changed "compiling" into "Compilation".

Comment: Li 259: brGDGTs concentrations?, fluxes? Data?
Response: It is the concentration of brGDGTs. We made change in revised manuscript.

Comment: Li 274-275: I would rephrase this section. Where is the statistical analysis?
Response: we wrote as "We further extend the dataset on global scale (Fig. 5), showing that the IIIa/IIa ratio is still significantly higher in marine sediments than soils ($p<0.01$)." Similar to Bohai Sea, we also perform statistical analysis but detailed results are not shown here.

Comment: Li 278: "unusually low" in which context are they low?
Response: Red Sea sediments showed unusually low IIIa/IIa values compared to other marine sediments. So we changed the sentence as "An exception was observed for Red Sea sediments which have unusually low IIIa/IIa values (0.39±0.21) compared to other marine sediments (>0.87)."

Comment: Li 279: "Bab el Mandeb" strait
Response: We corrected it.

Comment: Li 279: "litter" or low?
Response: we corrected this spelling error.

Comment: Li 280: salinity, no units?
Response: We added "PSU" after salinity.

Comment: Li 281-283: The Red Sea is an extreme environment?, the authors do not explain why the ratios from environments as different as those in Fig. 5 (e.g. Arctic, Mediterranean, Chilean margin, South China Sea, river waters and soils) fit within the scheme proposed to interpret the IIIa/IIa ratios, whereas the Red Sea does not. The interpretation proposed is not very convincing, particularly as they seem to argue through the text that the producers of brGDGTs in soils and marine settings are not the same type of organisms.
Response: we explained the reasons why Read Sea is different in brGDGTs' compositions from other marine systems. In the manuscript, we wrote as "the Red Sea has a restricted connection to the Indian Ocean via the Bab el Mandeb Strait. This, combined with high insolation, low precipitation and strong winds result in surface water salinity up to 41 PSU in the south and 36 PSU in the north of the Red Sea (Sofianos et al., 2002). Under such extreme environment, distinct microbial populations may be developed and produced GDGTs different from that in other marine settings (see Trommer et al., 2009 for details).

Comment: Li 284-286: level or values?
Response: we changed level into values in the revised manuscript.

Comment: Li 290: "Why do soils have lower IIIa/IIa" and the Red Sea?
Response: As we mentioned above, Red Sea is an exception. So we add "generally" in the sentence. Considering the comments from reviewer 1 and 2, we rewrote as "Why do marine sediments generally have higher IIIa/IIa values than soils?"

Comment: Li 302-303: Please explain further what is meant by and why is not related to the IIIa/IIa ratio: "because both soils and marine sediments are globally distributed and their temperatures (MAT vs. sea surface temperature) have no systematic difference".
Response: We accepted reviewer 1 and 2's suggestions, and rewrote the whole paragraph. Pleases see line 352 to 389 for details.

Comment: Li 305: "positive correlation with soil pH (R2=0.43)", really?, with such a R2 value?
Response: we already double check this point. In the revised manuscript, we added figure 6 that shows even higher R2 value between the IIIa/IIa ratio and soil pH (R2=0.51; Fig. 6) when global soil dataset are included.

Comment: Li 305-312: I would use more caution in this section as most of the evidence used to back the authors' interpretation is hypothetical
Response: we added this content in the revised manuscript as: "It should be pointed out that unlike fairly stable pH of overlying sea water, the pH of pore waters in marine sediments can vary significantly, which may influence compositions of brGDGTs. Nevertheless, at current stage, the occurrence of higher IIIa/IIa values in marine sediments is most likely attributed to relatively higher pH and lower deep water temperature. Further studies are needed to disentangle relative importance of these two factors."

Comment: Li 322-324: the regression in Fig. 6 is the product of wishful thinking. One can fit any curve to a group of unrelated data point and get "satisfactory" R2 value. I think that it is evident from Figure 6 that BIT and the III/II ratios are unrelated. There are two cluster of data. Why samples with BIT values below 0.3 (which are supposed to be only typical of sites with low terrigenous inputs) have such an spread of III/II values?, Similarly, how come that values of III/II

below 0.8, which are proposed to be only found in soils (li 285) has such an spread of BIT values from 0 to 1. It does not make sense to me if both indicators are indicators of marine vs. terrigenous organic carbon. Should not they fit into a simple straightforward linear regression if IIIa/IIa and BIT are both indexes for assessing soil organic carbon (inputs) in marine settings, as claimed by the authors? .
Response: we tried the different correlation between IIIa/IIa and BIT. The exponential correlation has much higher R2 value than linear correlation. This is the reason we showed exponential curve in figure 6 (figure 7 in revised manuscript). But more important, our key point is not correlation between IIIa/IIa and BIT. We want to show different clusters of marine and terrestrial samples based on these two indicators. We chose <0.3 and >0.67 as threshold values because they can include 90% of samples. We highlight this point in the manuscript.

Comment: Li 366-368: it is not necessary to say in the conclusions section that the authors have reached some conclusions. It is redundant.
Response: we delete this redundant sentences in revised manuscript.

Comment: Li 369: Please define what is meant by "generally lower", as it stands it is a subjective statement which is followed by values that are purported to reflect objective thresholds (which are not in fact).
Response: we changed

Comment: Li 369-370: The authors have not demonstrated the occurrence of terrestrial inputs in all samples studied (e.g. Fig. 6). They cannot claim that high values of III/II occur in sediments "devoid of significant terrestrial inputs". What is meant by significant anyhow?.
Response: Good comment. We rewrote as "Our investigation for brGDGTs in three Bohai Sea cores and globally distributed soils and marine sediments shows that the brGDGTs IIIa/IIa ratio is lower than 0.59 in 90% of soils, but higher than 0.92 in 90% of marine sediments devoid of significant terrestrial inputs"

Comment: Fig. 1: m/z of chrenarchaeol?
Response: We confirmed as crenarchaeol

Comment: Fig. 4 combines 4 graphs extracted from published papers that are unrelated to each other, and I think that they should go in different figures for coherence sake in the supplementary information section. Explain the abbreviations in the x-axis in fig. 4b in the legend.
Response: we combined the comments from reviewers 1 and 2 as well as Dr. Ding He. In order to express more clearly, we still leave the figure 4 in the manuscript.

Comment: Fig.5. The use of symbols of different size prevents the visualization of all the data in the map, as the big dots cover smaller dots, and also are easier to visualize that smaller dots, giving the impression that "there are more of them". Please use another way of visualizing all the data that provides equal weight to data with different range of values.
Response: we asked the suggestions from several senior scientists and biogeochemists. They think it is useful to use different size of symbol to reflect high or low values of IIIa/IIa. So we did not make any change in the revised manuscript.

Comment: Table 1: where are the samples from?. Please explain further what is mean by: "Different letters (a, b, c, d) represent significant difference at the level of $p<0.05$."
Response: We added more explanation in the title of Table 1.