An interlaboratory study of TEX$_{86}$ and BIT analysis of sediments, extracts, and standard mixtures

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[2] Two commonly used proxies based on the distribution of glycerol dialkyl glycerol tetraethers (GDGTs) are the TEX$_{86}$ (TetraEther indeX of 86 carbon atoms) paleothermometer for sea surface temperature reconstructions and the BIT (Branched Isoprenoid Tetraether) index for reconstructing soil organic matter input to the ocean. An initial round-robin study of two sediment extracts, in which 15 laboratories participated, showed relatively consistent TEX$_{86}$ values (reproducibility ±3–4°C when translated to temperature) but a large spread in BIT measurements (reproducibility ±0.41 on a scale of 0–1). Here we report results of a second round-robin study with 35 laboratories in which three sediments, one sediment extract, and two mixtures of pure, isolated GDGTs were analyzed. The results for TEX$_{86}$ and BIT index showed improvement compared to the previous round-robin study. The reproducibility, indicating interlaboratory variation, of TEX$_{86}$ values ranged from 1.3 to 3.0°C when translated to temperature. These results are similar to those of other temperature proxies used in paleoceanography. Comparison of the results obtained from one of the three sediments showed that TEX$_{86}$ and BIT indices are not significantly affected by interlaboratory differences in sediment extraction techniques. BIT values of the sediments and extracts were at the extremes of the index with values close to 0 or 1, and showed good reproducibility (ranging from 0.013 to 0.042). However, the measured BIT values for the two GDGT mixtures, with known molar ratios of crenarchaeol and branched GDGTs, had intermediate BIT values and showed poor reproducibility and a large overestimation of the "true" (i.e., molar-based) BIT index. The latter is likely due to, among other factors, the higher mass spectrometric response of branched GDGTs compared to crenarchaeol, which also varies among mass spectrometers. Correction for this different mass spectrometric response showed a considerable improvement in the reproducibility of BIT index measurements among laboratories, as well as a substantially improved estimation of molar-based BIT values. This suggests that standard mixtures should be used in order to obtain consistent, and molar-based, BIT values.

1. Introduction

[3] Reconstruction of ancient seawater temperatures is of considerable importance in understanding climate change. Over the past decades several geochemical temperature proxies have been developed to reconstruct past sea surface temperatures (SSTs) based on inorganic or organic fossil remains. Two of the most popular tools are the Mg/Ca ratio of planktonic foraminifera [*Nürnberg et al.*, 1996; *Elderfield and Ganssen*, 2000] and the U$^{k'}_{37}$ ratio based on long-chain C$_{37}$ alkenones derived from haptophyte algae [*Brassell et al.*, 1986; *Prahl and Wakeham*, 1987]. Another organic SST proxy is based on archaeal glycerol dialkyl glycerol tetraether (GDGT) lipids, the TEX$_{86}$ index [*Schouten et al.*, 2002]. These lipids are biosynthesized by marine archaea that synthesize GDGTs containing 0–3 cyclopentyl moieties (GDGT-0–GDGT-3; see structures in Figure 1). Members of the phylum Thaumarchaeota also synthesize crenarchaeol, a compound with a cyclohexyl moiety in addition to four cyclopentyl moieties (Figure 1), and smaller quantities of a crenarchaeol regioisomer (Cren'). The TEX$_{86}$ ratio was proposed as a mean to quantify the relative distribution of GDGTs [*Schouten et al.*, 2002]:

$$TEX_{86} = \frac{[GDGT\text{-}2] + [GDGT\text{-}3] + [Cren']}{[GDGT\text{-}1] + [GDGT\text{-}2] + [GDGT\text{-}3] + [Cren']} \quad (1)$$

[4] The TEX$_{86}$ index has been calibrated with annual-mean SST using marine sediment core tops [*Kim et al.*, 2008, 2010a]. This relationship has recently been reevaluated and two novel indices were proposed:

$$TEX^{H}_{86} = \log\left[\frac{[GDGT\text{-}2] + [GDGT\text{-}3] + [Cren']}{[GDGT\text{-}1] + [GDGT\text{-}2] + [GDGT\text{-}3] + [Cren']}\right] \quad (2)$$

$$TEX^{L}_{86} = \log\left[\frac{[GDGT\text{-}2]}{[GDGT\text{-}1] + [GDGT\text{-}2] + [GDGT\text{-}3]}\right] \quad (3)$$

[5] The TEX$^{H}_{86}$ is suggested to be applicable in warm, tropical regions, whereas TEX$^{L}_{86}$ is calibrated for cold, polar regions [*Kim et al.*, 2010a]. Besides SST reconstruction, the TEX$_{86}$ proxy is also used in some lakes to infer (paleo) surface water temperatures using a lake core top calibration [*Powers et al.*, 2004, 2010].

[6] In addition to archaeal GDGTs, bacterial GDGTs with nonisoprenoidal carbon skeletons are also frequently encountered in marine sediments (GDGT-I–GDGT-III, Figure 1). Several studies have shown that bacterial GDGTs are especially abundant in soils and peats [e.g., *Weijers et al.*, 2006] but decrease in marine sediments with increasing distance from the coast, suggesting a predominantly terrestrial origin [*Hopmans et al.*, 2004; *Herfort et al.*, 2006; *Kim et al.*, 2006]. *Hopmans et al.* [2004] proposed the BIT index to quantify the abundance of these bacterial GDGTs relative to crenarchaeol as a proxy for the input of terrestrial organic matter (OM) into marine sediments:

$$BIT = \frac{[GDGT\text{-}I] + [GDGT\text{-}II] + [GDGT\text{-}III]}{[Crenarchaeol] + [GDGT\text{-}I] + [GDGT\text{-}II] + [GDGT\text{-}III]} \quad (4)$$

[7] Subsequent studies have shown that this proxy can be applied to trace soil OM in coastal marine environments [e.g., *Huguet et al.*, 2007; *Walsh et al.*, 2008; *Smith et al.*, 2010] as long as the export fluxes of crenarchaeol to sediments are comparable among sites [*Fietz et al.*, 2011; *Smith et al.*, 2012] and the relative proportions of crenarchaeol versus marine OM and branched GDGTs versus soil OM remain constant. Furthermore, *Weijers et al.* [2006] found that a high input of soil OM in marine sediments can potentially bias TEX$_{86}$ values as soils can also contribute

GDGT-0 ([M+H]$^+$=1302)

GDGT-1 ([M+H]$^+$=1300)

GDGT-2 ([M+H]$^+$=1298)

GDGT-3 ([M+H]$^+$=1296)

Crenarchaeol ([M+H]$^+$=1292)

GDGT-I ([M+H]$^+$=1022)

GDGT-II ([M+H]$^+$=1036)
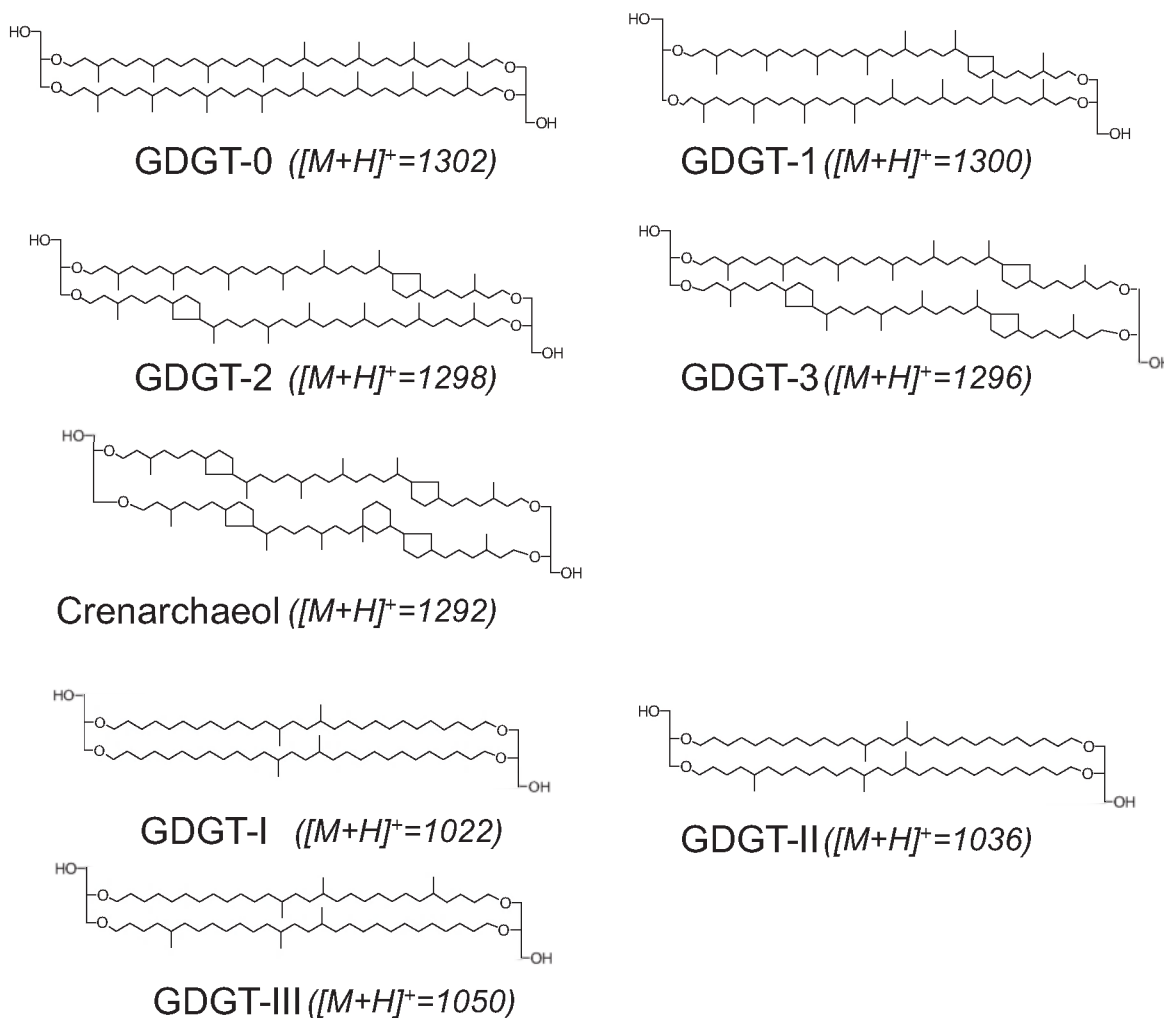
GDGT-III ([M+H]$^+$=1050)

Figure 1. Structures and [M+H]$^+$ protonated molecules of GDGTs analyzed in the round-robin study.

isoprenoidal GDGTs. They recommended simultaneous reporting of BIT indices in order to monitor for this effect. BIT values can range from 0.01 in open marine sediments to 1 in some soils [*Schouten et al.*, 2013, and references cited therein].

[8] A prerequisite for the wider application of geochemical proxies is the robustness of analytical reproducibility. GDGTs are analyzed by high-performance liquid chromatography (HPLC) coupled to mass spectrometry (MS) [*Hopmans et al.*, 2000; *Schouten et al.*, 2007; *Escala et al.*, 2007]. A common procedure to validate analytical methodology and help laboratories detect and remediate inaccuracies in their results is to conduct a round-robin test (also known as proficiency test or interlaboratory comparison) [*Thompson et al.*, 2006], as has been done for the U$_{37}^{k'}$ ratio of long-chain C$_{37}$ alkenones [*Rosell-Melé et al.*, 2001] and

for the Mg/Ca ratio of (foraminiferal) carbonates [*Rosenthal et al.*, 2004; *Greaves et al.*, 2008]. An initial round-robin study for TEX$_{86}$ and BIT analyses was performed in 2008 [*Schouten et al.*, 2009] on filtered polar fractions obtained from extracts of two sediments. For TEX$_{86}$ the repeatability (i.e., intralaboratory variation) was 0.028 and 0.017, respectively, for the two sediment extracts. This translates to ±1–2°C of calculated temperature variation using contemporary TEX$_{86}$-SST calibrations. The reproducibility, indicating interlaboratory variation, of TEX$_{86}$ measurements was substantially higher: 0.050 and 0.067, respectively, or ±3–4°C when translated to temperature. These temperature uncertainties were higher than those obtained in round-robin studies of the Mg/Ca (2–3°C) and U$_{37}^{k'}$ (1–2°C) paleothermometers and suggest relatively large variations between laboratories in TEX$_{86}$ measurements. Repeatability of BIT measurements

for the sediment extract with substantial amounts of soil OM input was relatively small, 0.029, but reproducibility between laboratories was large, 0.410 on a scale of 0–1. This large dispersion was attributed to the large structural differences between the GDGTs used in the BIT index (e.g., the higher-molecular-weight crenarchaeol versus the lower-molecular-weight branched GDGTs), which may give rise to variable responses in the different mass spectrometers used.

[9] Here we describe the results of an anonymized second round-robin study involving 35 laboratories. The six samples distributed consisted of three different homogenized sediments, one sediment extract, and two mixtures of isolated branched GDGTs and crenarchaeol mixed in known, pre-weighed quantities. The results shed light on the effect of extraction and separation techniques on the analyses of TEX$_{86}$ and BIT indices of sediments as well as on the necessity of using standard mixtures to calibrate the BIT index.

2. Materials and Methods

[10] A general invitation was sent to a large number of laboratories to participate in an anonymous round-robin study, to which 36 laboratories responded positively. These laboratories received three 60 mL vials containing homogenized sediment (labeled "Sediments A, B, or C") prepared at Harvard University and at the Royal Netherlands Institute for Sea Research (NIOZ) and three 3 mL vials containing mixtures of organic compounds or extracts (labeled "organic fractions D, E, and F") prepared at the NIOZ. Laboratories were requested to analyze the samples when their HPLC-MS setup was performing well according to their criteria and to analyze sediments at least in triplicate and the organic fractions at least fivefold. The vials were distributed at the start of November 2011, and results reported here are those of the 35 laboratories, which reported their results before 1 April 2012 (see supporting information).[1] Results reported after the deadline were not considered in this study. The study was performed "doubly blind," i.e., statistical treatment of the results was performed by individuals (A.R.M. and S.S.) unaware of the contents of the round-robin samples (prepared by A.P. and E.C.H.), and identities of the laboratories were anonymized by Lloyd

Snowdon (Department of Geoscience, University of Calgary, Canada), who was not involved in analysis or statistical treatment of the data.

2.1. Sediment Origin

[11] Sediment A was obtained from Salt Pond, Falmouth, Massachusetts, USA (41°32′N, 70°37′W; water depth 3 m). In July of 2011, 33 kg of wet sediment was homogenized, dried, pulverized, and rehomogenized. This yielded 4 kg dry sediment, of which ca. 500 g was aliquoted in fifty 60 mL amber glass vials in 10 g portions.

[12] Sediment B was obtained from a 46 kg box core from the Carolina Margin (35°50′N, 74°50′W; water depth ca. 600 m) in July of 1996 and stored frozen at −20°C. In August of 2011 it was thawed and processed analogously to the Salt Pond sample, yielding 18.2 kg dry sediment, of which ca. 1 kg was aliquoted in fifty 60 mL amber glass vials in 20 g portions.

[13] Sediment C was derived from the upper part of a piston core (TY92-310G; 16°03′N, 52°71′E; 880 m water depth; 0 to 42 cm depth) taken in the Arabian Sea. The sediment was freeze-dried and ground using mortar and pestle to obtain 1.5 kg of dry sediment. Approximately 500 g of the sediment was extracted to obtain "organic fraction F" (see below), while 1 kg of sediment was aliquoted in fifty 60 mL amber glass vials in 20 g portions.

2.2. Preparation of Extract and Mixtures

[14] To obtain organic fraction F, Sediment C was divided in several aliquots and extracted using an Automated Solvent Extractor (ASE) 200, DIO-NEX, 100°C, and 7.6×10^6 Pa with a mixture of dichloromethane (DCM):methanol (MeOH) (9:1, vol/vol). Total lipid extracts were separated over a column filled with aluminum oxide into apolar and polar fractions using hexane:DCM (9:1, vol/vol) and DCM:MeOH (1:1, vol/vol), respectively. Polar fractions were combined, condensed by rotary evaporation, dried under a stream of nitrogen, and weighed and dissolved in hexane/isopropanol (99:1, vol/vol) in a concentration of 2 mg mL^{-1}. Aliquots of 0.5 mg of polar fraction (labeled organic fraction F) were filtered using a PTFE (Polytetrafluoroethylene) 0.4 μm filter, dried under a stream of nitrogen, and placed in fifty 3 mL vials.

[15] Organic fractions D and E contained mixtures of three isolated GDGT standards: crenarchaeol, GDGT-I, and GDGT-II. The branched GDGTs

Table 1. Sample Work Up of Sediments[a]

Laboratory Number	Extraction	Solvent	Treatment	Column Fraction
1	ASE	DCM:MeOH, 9:1	Al$_2$O$_3$ column	DCM:MeOH, 1:1
2	ASE	DCM:MeOH, 9:1	Al$_2$O$_3$ column	DCM:MeOH, 1:1
3	Ultrasonic	DCM:MeOH, 1:1	None	
4	Ultrasonic	H$_2$O:MeOH:DCM, 4:10:5	Al$_2$O$_3$ column	DCM:MeOH, 1:1
5	Microwave	DCM:MeOH, 3:1	SiO$_2$ column	MeOH
6	Ultrasonic	DCM:MeOH, 9:1	Al$_2$O$_3$ column	DCM:MeOH, 1:1
7	Soxhlet	MeOH:DCM, 9:1	SiO$_2$ column	DCM:MeOH, 1:1
8	ASE	DCM:MeOH, 9:1	Al$_2$O$_3$ column	DCM:MeOH, 1:1
9	Ultrasonic	DCM:MeOH, 3:1	Hydrolysis, SiO$_2$ column	DCM:MeOH, 1:1
10	Microwave	DCM:MeOH, 9:1	SiO$_2$ column	DCM:MeOH, 1:1
11	ASE	DCM:MeOH, 9:1	SiO$_2$ column	MeOH
12	Ultrasonic	DCM:MeOH, 2:1	Al$_2$O$_3$ column	DCM:MeOH, 1:1
13	ASE	DCM:MeOH, 9:1	Al$_2$O$_3$ column	DCM:MeOH, 1:1
14	n.r.			
15	Ultrasonic	MeOH, MeOH:DCM (1:1), DCM	SiO$_2$ column	MeOH
16	Ultrasonic	CHCl$_3$:MeOH:ammonium acetate buffer, 2:1:0.8		
17	Ultrasonic	MeOH, MeOH:DCM (1:1), DCM	Hydrolysis, Al$_2$O$_3$ column	DCM:MeOH, 1:1
18	ASE	DCM:MeOH, 6:4	SiO$_2$ column	Toluene:MeOH, 3:1
19	ASE	DCM:MeOH, 9:1	SiO$_2$ column	DCM:MeOH, 1:1
20	ASE	DCM:MeOH, 9:1	Al$_2$O$_3$ column	DCM:MeOH, 1:1
21	ASE	DCM:MeOH, 93:7	Al$_2$O$_3$ column	DCM:MeOH, 1:1
22	ASE	DCM:MeOH, 9:1	Al$_2$O$_3$ column	DCM:MeOH, 1:1
23	ASE	DCM:MeOH, 9:1	None	
24	Ultrasonic	MeOH, MeOH:DCM (1:1), DCM	SiO$_2$ column	DCM:MeOH, 1:1
25	Microwave	DCM:MeOH, 3:1	Hydrolysis	None
26	ASE	DCM:MeOH, 9:1	Al$_2$O$_3$ column	DCM:MeOH, 1:1
27	ASE	DCM:MeOH, 9:1	Al$_2$O$_3$ column	DCM:MeOH, 1:1
28	ASE/Soxhlet	DCM:MeOH, 2:1	SiO$_2$/Al$_2$O$_3$ column	DCM:MeOH, 1:1
29	Ultrasonic	MeOH, MeOH:DCM (1:1), DCM	H$_2$O extraction of Hex:IPa	
30	Ultrasonic	CHCl$_3$:MeOH:phosphate buffer, trichloroacetic acid	None	
31	ASE	DCM:MeOH, 9:1	Al$_2$O$_3$ column	DCM:MeOH, 1:1
32	n.r.			
33	Microwave	DCM:MeOH, 9:1	Hydrolysis, NH$_2$-SiO$_2$ column	DCM:acetone, 9:1
34	Ultrasonic	CHCl$_3$:MeOH:phosphate buffer, 2:1:0.8	None	
35	Soxhlet	DCM:MeOH, 2:1	Al$_2$O$_3$ column	DCM:MeOH, 1:2

[a]ASE, accelerated solvent extraction; n.r., not reported.

were isolated from a large extract of sediment derived from a piston core taken in the Drammensfjord, Norway (D2-H; 59°40.11'N, 10°23.76'E; water depth 113 m; sediment depth 746–797 cm), while crenarchaeol was isolated from the remainder of Sediment C. The sediments were Soxhlet-extracted (24 h) using a mixture of DCM and MeOH (7:1, vol/vol). The combined extracts were separated over a column filled with aluminum oxide into an apolar and polar fraction using hexane:DCM (9:1, vol/vol) and DCM: MeOH (1:1, vol/vol), respectively. GDGTs were first isolated in two stages using normal phase HPLC followed by flow injection analysis according to *Smittenberg et al.* [2002]. Columns used were a semipreparative and an analytical Alltech Prevail Cyano column (250 mm × 10 mm, 5 μm, and flow rate 3 mL min^{-1} and 250 mm × 4.6 mm, 5 μm, and flow rate 1 mL min^{-1}, respectively). The isolated GDGTs were further cleaned using reversed phase chromatography modified from

Ingalls et al. [2006]. Briefly, GDGTs were dissolved in ethyl acetate and injected onto a Zorbax Eclipse XDB C-8 column (4.6 mm × 150 mm; 5 μm; Agilent Technologies). GDGTs were eluted with the following program with acetonitrile (A) and ethyl acetate (B) as mobile phase: 0–10% B in 4 min, 10–35% B in 10 min, 35–69% B in 6 min, 69–100% B in 7 min, with a flow rate of 1 mL min^{-1}. This yielded 5.0 mg of crenarchaeol, 3.8 mg of GDGT-I, and 3.7 mg of GDGT-II. The purity of the GDGTs was first assessed by full scan (m/z 300–2000) HPLC-atmospheric pressure chemical ionization (APCI)/MS, which did not reveal major fragments other than those from the GDGTs [cf. *Hopmans et al.*, 2000]. In addition, we performed ^{1}H and ^{13}C NMR analyses on the purified compounds. All the major signals of the GDGTs could be assigned [see *Schouten et al.*, 2013, Figure 7]. Only in the case of GDGT-II some minor signals were found that could not be attributed to GDGTs. Although the exact purity

Table 2. HPLC-MS Methods Reported by Participants in the Round-Robin Study[a]

Laboratory Number	HPLC Column	HPLC Gradient	MS	MS Type	MS Method	Integration
1	Prevail Cyano	Hex:IPA	Shimadzu 2010A	Single quad	SIM	$[M+H]^+$ ions
2	Prevail Cyano	Hex:IPA	PE Sciex API 300	Single quad	Mass scanning	$[M+H]^+$ ions
3	Prevail Cyano	Hex:IPA	Bruker Esquire 3000+	Ion trap	Mass scanning	$[M+H]^+$ ions
4	Prevail Cyano	Hex:IPA	Agilent 6130	Single quad	SIM	$[M+H]^+$ ions
5	Tracer Excel CN	Hex:IPA	Thermo TSQ Quantum	Triple quad	SIM	$[M+H]^+$ ions
6	Prevail Cyano	Hex:IPA	Agilent 6460A	Triple quad	SIM	$[M+H]^+$ ions
7	Prevail Cyano	Hex:IPA	Agilent XCT (Bruker)	Ion trap	Mass scanning	n.r.
8	Prevail Cyano	Hex:IPA	Bruker HCTUltra ETD II	Ion trap	Mass scanning	$[M+H]^+$ ions
9	Prevail Cyano	Hex:IPA	Micromass Quattro Ultima	Triple quad	SIM	$[M+H]^+$ ions
10	Prevail Cyano	Hex:IPA	Agilent 6130	Single quad	SIM	$[M+H]^+$ ions
11	Prevail Cyano	Hex:IPA	Agilent 6130	Single quad	SIM	$[M+H]^+$ ions
12	Prevail Cyano	Hex:IPA	Agilent 6120	Single quad	SIM	$[M+H]^+$ ions
13	Prevail Cyano	Hex:IPA	Thermo LCQ Deca XP	Ion trap	"SIM"	$[M+H]^+$ ions
14	n.r.					
15	Prevail Cyano	Hex/DCM:IPA	Agilent 6120	Single quad	SIM	$[M+H]^+$ ions
16	Prevail Cyano	Hex:IPA	n.r.	Ion trap	"SIM"	$[M+H]^+$, +1 ions
17	Prevail Cyano	Hex:IPA	Agilent 6410	Triple quad	SIM	$[M+H]^+$ ions
18	Prevail Cyano	Hex:IPA	Bruker Daltonics µTOF	TOF-MS	Mass scanning	$[M+H]^+$, +1 ions
19	Prevail Cyano	Hex:IPA	Thermo LSQ Fleet	Ion trap	n.r.	n.r.
20	Prevail Cyano	Hex:IPA	Agilent 6460	Triple quad	SIM	$[M+H]^+$ ions
21	Prevail Cyano	Hex:IPA	Micromass Quattro Ultima	Triple quad	SIM	$[M+H]^+$ ions
22	Prevail Cyano	Hex:IPA	Thermo LTQ Orbitrap XL	Orbitrap	Mass scanning	$[M+H]^+$ ions
23	Prevail Cyano	Hex:IPA	Thermo TSQ Quantum	Triple quad	SIM	$[M+H]^+$, +1 ions
24	Prevail Cyano	Hex:IPA	Agilent 6460	Triple quad	SIM	$[M+H]^+$ ions
25	Prevail Cyano	Hex:IPA	Thermo LSQ	Ion trap	Mass scanning	$[M+H]^+$, +1 ions
26	Prevail Cyano	Hex:IPA	Agilent 6130	Single quad	SIM	$[M+H]^+$ ions
27	Prevail Cyano	Hex:IPA	Agilent 1100	Single quad	SIM	$[M+H]^+$ ions
28	Prevail Cyano	Hex:IPA	Agilent 6130	Single quad	SIM	$[M+H]^+$ ions
29	Prevail Cyano	Hex:IPA	Agilent 1100	Single quad	SIM	$[M+H]^+$ ions
30	Prevail Cyano	Hex:IPA	Agilent 6130	Single quad	SIM	$[M+H]^+$ ions
31	Prevail Cyano	Hex:IPA	Agilent 1100 SL	Single quad	SIM	$[M+H]^+$ ions
32	Prevail Cyano	Hex:IPA	Agilent 1200 SL	Single quad	SIM	$[M+H]^+$ ions
33	Prevail Cyano	Hex:IPA	Agilent 1200	Single quad	SIM	$[M+H]^+$ ions
34	Prevail Cyano	Hex:IPA	Agilent 1200 SL	Single quad	SIM	$[M+H]^+$ ions
35	Prevail Cyano	Hex:IPA	Thermo TSQ Quantum	Triple quad	SIM	$[M+H]^+$ ions

[a]Hex, hexane; IPA, isopropanol; DCM, dichloromethane; SIM, selected ion monitoring; TOF, time of flight; n.r., not reported.

cannot be assessed, the MS and NMR data suggest that GDGTs are likely to be more than ∼90% pure. Isolated GDGTs were weighed on a microbalance (accuracy 0.1 µg), and two mixtures were prepared, Mixtures D and E. Each vial of Mixture D contained 500 µL, pipetted using a positive displacement micropipette with precision of 0.5%, of a 25 mL solution containing 200 µg of crenarchaeol, 50 µg of branched GDGT-II, and 75 µg of branched GDGT-I. The final ratio of GDGTs was 200:50:75 (wt/wt/wt) leading to a mass-based "BIT" value (BIT$_{mass}$) of 0.385 and a molar-based "BIT" value (BIT$_{mol}$) of 0.440. Each vial of Mixture E contained 500 µL of a 25 mL solution containing 1000 µg of isolated crenarchaeol, 100 µg of isolated branched GDGT-II, and 50 µg of isolated branched GDGT-I. The final ratio of GDGTs was 100:10:5 (wt/wt/wt) leading to a BIT$_{mass}$ of 0.130 and a BIT$_{mol}$ value of 0.158. Uncertainties in the weighing of compounds, pipetting of mixtures, and compound purity lead to an uncertainty of ∼0.01 in BIT values.

2.3. TEX$_{86}$ and BIT Analyses

[16] The sediments were extracted and fractionated according to the protocols used by each individual laboratory (Table 1). GDGT Mixtures D and E and Extract F were analyzed as provided. All laboratories used HPLC/APCI/MS to analyze GDGTs (Table 2).

2.4. Statistical Analysis

[17] The reporting and analysis of the data were performed following some of the recommendations of the IUPAC (International Union of Pure and Applied Chemistry, Basel, Switzerland) for the proficiency testing of analytical chemistry laboratories [*Thompson et al.*, 2006]. The results, i.e., the laboratory means, were assessed using histograms and Tukey box plots. The latter were used as the only means employed to identify outliers, which are those data that fall beyond the whiskers (±1.5 times the difference between the 3rd and 1st quartiles or the interquartile range which includes

Table 3. Reported Results of TEX$_{86}$ Analysis of Sediments A, B, and C and Extract F[a]

Laboratory Number	TEX$_{86}$ A	SD	n	TEX$_{86}$ B	SD	n	TEX$_{86}$ C	SD	n	TEX$_{86}$ F	SD	n
1	0.426	0.042	2	0.510	0.024	3	0.681	0.014	3	0.681	0.006	8
2	0.603	0.006	3	0.487	0.006	3	0.740	0.010	3	0.740	0.007	5
3	0.562	0.013	3	0.520	0.005	3	0.686	0.002	3	0.697	0.017	6
4	0.589	0.004	3	0.562	0.008	3	0.724	0.005	3	0.708	0.004	5
5	0.574	0.001	3	0.602	0.001	3	0.676	0.002	3	0.677	0.005	3
6	0.590	0.004	3	0.536	0.002	3	0.694	0.003	3	0.685	0.002	5
7	0.540	0.009	3	0.553	0.005	3	0.716	0.001	3	0.708	0.008	5
8	0.577	0.013	3	0.541	0.013	3	0.696	0.002	3	0.695	0.003	5
9	0.414	0.006	3	0.505	0.001	3	0.662	0.003	3	0.682	0.002	3
10	0.586	0.003	3	0.542	0.005	3	0.710	0.003	3	0.707	0.006	3
11	0.490	0.000	3	0.583	0.006	3	0.740	0.000	3	0.720	0.000	5
12	0.504	0.009	3	0.546	0.012	3	0.709	0.010	3	0.710	0.004	7
13	0.497	0.000	1	0.476	0.000	1	0.637	0.000	1	0.637	0.015	4
14	0.453	0.000	1	0.560	0.000	1	0.695	0.000	1	0.699	0.014	3
15	0.552	0.009	3	0.527	0.006	3	0.680	0.002	3	0.679	0.002	5
16	n.r.			0.473	0.006	3	0.648	0.003	3	0.645	0.003	4
17	0.491	0.019	3	0.558	0.021	3	0.726	0.003	3	0.738	0.002	3
18	0.590	0.002	2	0.547	0.009	3	0.711	0.002	3	0.722	0.003	6
19	0.529	0.002	3	0.545	0.001	3	0.707	0.002	3	0.702	0.002	3
20	0.547	0.010	3	0.549	0.002	3	0.730	0.021	3	0.740	0.095	3
21	0.481	0.005	3	0.514	0.011	3	0.664	0.004	3	0.668	0.004	5
22	n.r.			0.575	0.000	1	0.687	0.000	1	0.685	0.003	5
23	0.528	0.043	3	0.543	0.017	3	0.701	0.002	3	0.682	0.007	3
24	0.573	0.014	2	0.547	0.014	2	0.710	0.000	2	0.716	0.005	5
25	0.588	0.011	3	0.573	0.019	3	0.701	0.009	3	0.706	0.002	5
26	0.553	0.031	3	0.568	0.004	3	0.728	0.003	3	0.729	0.004	7
27	0.557	0.037	3	0.538	0.004	3	0.709	0.001	3	0.693	0.005	5
28	0.579	0.006	3	0.544	0.011	3	0.703	0.009	3	0.704	0.003	3
29	0.520	0.030	3	0.636	0.011	3	0.723	0.009	3	0.717	0.015	3
30	0.464	0.005	3	0.535	0.003	3	0.702	0.002	3	0.701	0.001	5
31	0.574	0.005	3	0.520	0.000	3	0.692	0.002	3	0.692	0.004	5
32	0.646	0.006	3	0.563	0.003	3	0.724	0.002	3	0.717	0.003	3
33	0.497	0.004	3	0.574	0.002	3	0.731	0.001	3	0.726	0.006	6
34	0.610	0.014	3	0.569	0.048	3	0.729	0.003	3	0.750	0.006	5
35	0.533	0.009	3	0.577	0.003	3	0.704	0.002	3	0.704	0.007	5

[a]n.r., not reported.

25% of all data higher and 25% of all the data lower than the median). Performance of each lab was assessed using the Z-score, which is a measure of the distance between their data and the community mean (see Table S1). In addition, a one-way analysis of variance test was used to analyze the means and calculate the interlaboratory and the intralaboratory variance. Outliers were removed before these calculations. Values of variance have also been expressed in the form of relative standard deviation (equivalent to coefficient of variation, in percentage units). The reproducibility (s_R) is the interlaboratory precision, and the repeatability (s_r) is an estimate of the reliability of a method for a particular laboratory [*Nilsson et al.*, 1997] that reflects the precision of the analysis of replicate test samples. The repeatability and reproducibility values have also been expressed using confidence intervals as recommended by the ISO 5725 guidelines [*International Organization for Standardization*, 1986].

3. Results and Discussion

[18] The results of the TEX$_{86}$ and BIT analyses of the different laboratories are listed in Tables 3 and 4, respectively, and plotted in Figures 2 and 3, respectively, while the extraction methods and HPLC-MS conditions used are summarized in Tables 1 and 2, respectively. The most common method involved extractions using a DCM/MeOH mixture, typically followed by some form of column chromatography (Table 1). The HPLC methods used by the different laboratories are listed in Table 2 and nearly all were similar to that of *Schouten et al.* [2007] (i.e., a cyano column eluted with a gradient of isopropanol in hexane). However, a variety of mass spectrometry techniques were used: 24 laboratories used quadrupole-MS (15 used a single quadrupole and 9 used a triple quadrupole in single quadrupole mode), 7 laboratories used ion trap-MS, 1 laboratory used a time-of-flight-MS, and 1 laboratory used an Orbitrap

Table 4. Reported Results of BIT Index Analysis for Sediments A, B, and C, the GDGT Mixtures D and E, and Extract F

Laboratory Number	BIT A	SD	n	BIT B	SD	n	BIT C	SD	n	BIT D	SD	n	BIT E	SD	n	BIT F	SD	n
1	0.975	0.010	3	0.285	0.147	3	0.074	0.000	1	0.827	0.012	7	0.670	0.008	5	0.085	0.006	8
2	0.957	0.006	3	0.267	0.006	3	0.247	0.006	3	0.830	0.000	5	0.706	0.009	5	0.296	0.009	5
3	0.897	0.009	3	0.021	0.000	3	0.004	0.000	3	0.390	0.023	6	0.136	0.013	6	0.004	0.000	6
4	0.972	0.004	3	0.156	0.022	3	0.043	0.006	3	0.742	0.004	5	0.458	0.004	5	0.048	0.004	5
5	0.966	0.001	3	0.124	0.013	3	0.033	0.001	3	0.711	0.022	3	0.438	0.019	3	0.066	0.002	3
6	0.945	0.002	3	0.092	0.005	3	0.028	0.001	3	0.689	0.002	5	0.374	0.003	5	0.047	0.001	5
7	0.904	0.003	3	0.028	0.001	3	0.007	0.000	3	0.406	0.006	5	0.155	0.008	5	0.003	0.000	5
8	0.957	0.006	3	0.067	0.001	3	0.036	0.001	3	0.610	0.036	5	0.354	0.002	5	0.038	0.000	5
9	0.940	0.003	3	0.075	0.003	3	0.023	0.000	3	0.612	0.003	3	0.330	0.005	3	0.021	0.001	3
10	0.926	0.022	3	0.067	0.006	3	0.020	0.000	3	0.620	0.000	3	0.280	0.000	3	0.020	0.000	3
11	0.943	0.006	3	0.070	0.000	3	0.020	0.000	3	0.602	0.008	5	0.322	0.004	5	0.026	0.005	5
12	0.963	0.001	3	0.110	0.002	3	0.034	0.005	3	0.754	0.005	5	0.441	0.019	5	0.021	0.002	7
13	0.951	0.000	1	n.r.			n.r.			n.r.			n.r.			n.r.		
14	0.967	0.003	3	0.097	0.000	1	0.036	0.000	1	0.695	0.005	4	0.429	0.009	3	0.034	0.002	3
15	0.974	0.003	3	0.126	0.005	3	0.055	0.002	3	0.765	0.013	3	0.556	0.035	3	0.061	0.002	5
16	n.r.			0.157	0.002	3	0.079	0.004	3	0.785	0.002	3	0.582	0.006	3	0.104	0.001	4
17	0.920	0.008	2	0.149	0.016	3	0.043	0.002	3	0.757	0.001	3	0.428	0.003	3	0.040	0.001	3
18	0.971	0.001	3	0.033	0.003	3	0.008	0.000	3	0.461	0.004	6	0.200	0.005	6	0.021	0.005	6
19	0.960	0.007	3	0.122	0.002	3	0.026	0.002	3	0.650	0.003	3	0.329	0.002	3	0.024	0.001	3
20	0.970	0.000	3	0.111	0.019	3	0.030	0.000	3	0.740	0.104	3	0.370	0.017	3	0.037	0.006	3
21	0.961	0.000	1	0.199	0.002	3	0.081	0.002	3	0.814	0.011	5	0.540	0.007	5	0.080	0.000	5
22	n.r.			0.151	0.000	1	0.060	0.000	1	0.698	0.008	6	0.433	0.007	5	0.058	0.008	5
23	0.942	0.019	3	0.114	0.002	3	0.033	0.001	3	0.719	0.006	3	0.425	0.005	3	0.058	0.003	3
24	0.972	0.012	2	0.093	0.005	2	0.028	0.002	2	0.758	0.004	5	0.542	0.004	5	0.020	0.000	5
25	0.917	0.002	3	0.049	0.001	3	0.011	0.001	3	0.504	0.005	5	0.216	0.001	5	0.014	0.000	5
26	0.963	0.003	3	0.116	0.001	3	0.031	0.002	3	0.738	0.008	5	0.420	0.000	5	0.040	0.010	7
27	0.930	0.006	3	0.054	0.002	3	0.014	0.001	3	0.539	0.005	5	0.247	0.003	5	0.019	0.001	5
28	0.941	0.007	3	0.070	0.026	3	0.017	0.002	3	0.609	0.001	3	0.372	0.001	3	0.022	0.007	3
29	0.990	0.000	3	0.284	0.036	3	0.181	0.052	3	0.823	0.006	3	0.637	0.015	3	0.183	0.006	3
30	0.957	0.002	3	0.126	0.034	3	0.037	0.002	3	0.708	0.001	5	0.393	0.006	5	0.040	0.001	5
31	0.940	0.000	3	0.060	0.000	3	0.010	0.000	3	0.630	0.000	5	0.334	0.005	5	0.010	0.000	5
32	0.959	0.004	3	0.110	0.004	3	0.040	0.001	3	0.719	0.001	3	0.393	0.001	3	0.034	0.000	3
33	0.957	0.001	3	0.093	0.001	3	0.024	0.001	3	0.718	0.003	5	0.542	0.008	5	0.024	0.002	6
34	0.977	0.000	3	0.114	0.005	3	0.027	0.001	3	0.769	0.009	5	0.361	0.007	5	0.025	0.001	5
35	0.932	0.002	3	0.069	0.001	3	0.024	0.001	3	0.579	0.001	5	0.291	0.009	5	0.031	0.004	5

MS. Note that most laboratories analyzed the samples within 1–2 days, and thus the reported standard deviations do not represent the long-term laboratory repeatability.

3.1. TEX₈₆ Analysis of Sediments and Extract

[19] The results of the TEX₈₆ analysis are listed in Table 3, summarized in Table 5, and shown in Figure 2. Sediment C is from a tropical marine environment, the Arabian Sea, while Sediments A and B are from a temperate lake (Salt Pond, USA) and coastal shelf (Carolina Margin), respectively. These different environments are well reflected in the TEX₈₆ values, which are substantially higher (mean value 0.702, median value 0.704) for the tropical sediment than for the sediments from the temperate environments (mean 0.540 and 0.546, respectively, and median 0.552 and 0.546, respectively; Table 5). The results have a reasonably Gaussian-like distribution (Figure 4), with smaller

ranges in TEX₈₆ values for Sediment C and Extract F compared to Sediments A and B. We statistically identified (see section 2.4) one outlier for Sediment B (Laboratory 29), which was removed from subsequent statistical treatment.

[20] The estimated repeatability for TEX₈₆, after removal of the outlier, ranged from 0.006 to 0.016 (Table 5). Reproducibility, however, is larger and ranged from 0.023 to 0.053. The better reproducibility and repeatability of Sediment C is probably due to the higher abundances of the minor GDGTs, GDGT-1–GDGT-3 and the crenarchaeol regioisomer, relative to GDGT-0 and crenarchaeol. This likely has enabled a more reliable quantification of these minor compounds, as amounts were not only above the limit of detection but also above the limit of quantification, which is probably an order of magnitude higher than the limit of detection [cf. *Schouten et al.*, 2007]. Oddly enough, Extract F, the extract of Sediment C, has a somewhat worse repeatability and reproducibility
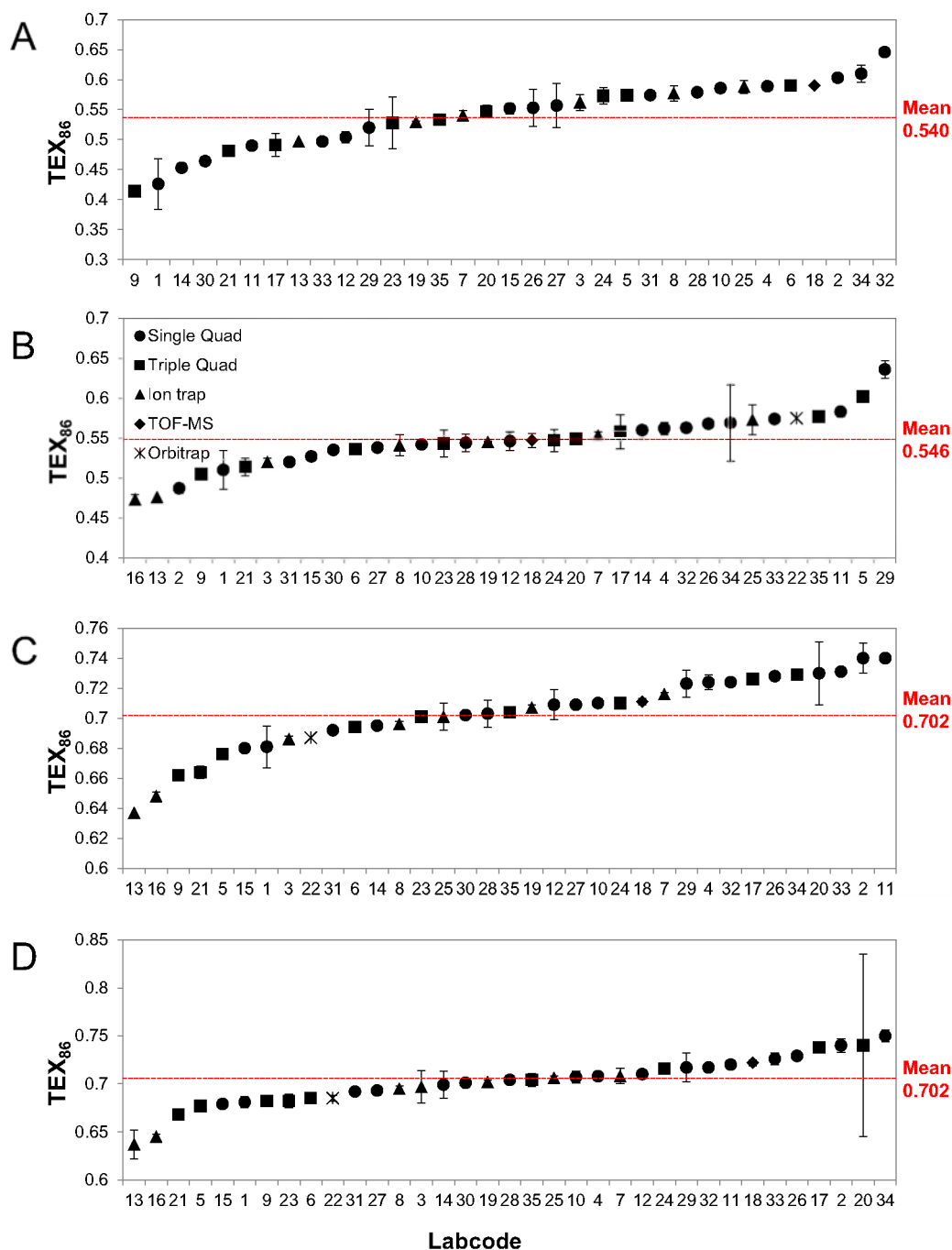
Figure 2. Individual TEX$_{86}$ values per laboratory of (a) Sediment A, (b) Sediment B, (c) Sediment C, and (d) Extract F. The dotted lines are the means of means reported by individual laboratories.

than the results of the sediment, in part due to the poor repeatability of the measurement of Extract F by Laboratory 20 (Figure 2d).

[21] If we convert these TEX$_{86}$ values to temperatures (based on *Kim et al.* [2008], rather than *Kim et al.* [2010a], to allow comparison with the round-robin results of *Schouten et al.* [2009]), then

the repeatability of TEX$_{86}$ analysis ranges from 0.4 to 0.9°C, while the reproducibility ranges from 1.3 to 3.0°C. This performance is better than the initial round-robin exercise of *Schouten et al.* [2009]. There, for the two samples (an Arabian Sea sediment extract and a Drammensfjord sediment extract) the estimated repeatability was
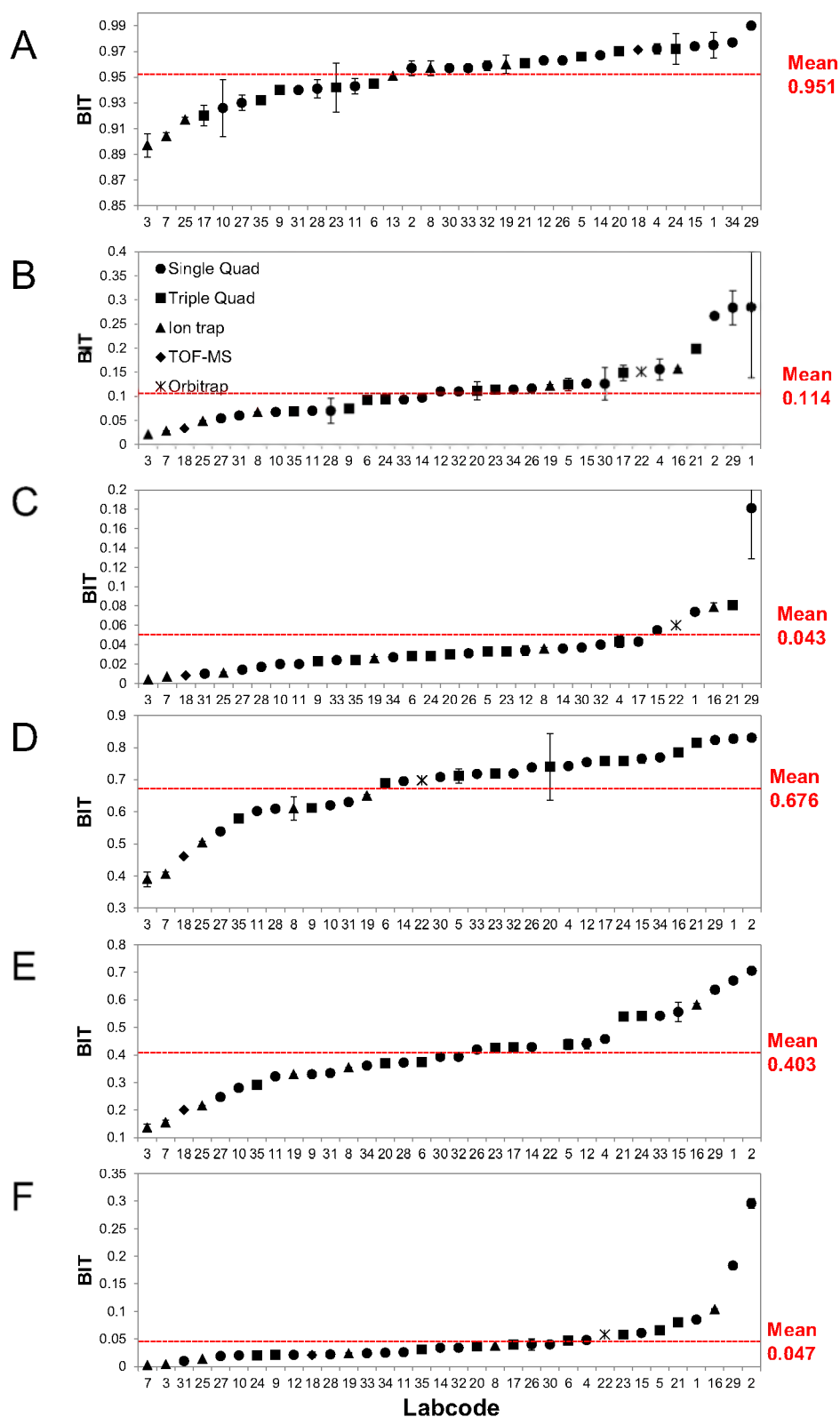
Figure 3. Individual BIT index values per laboratory of (a) Sediment A, (b) Sediment B, (c) Sediment C, (d) GDGT Mixture D, (e) GDGT Mixture E, and (f) Extract F. The dotted lines are the means of means reported by individual laboratories.

Table 5. Summary Statistics of TEX$_{86}$ Analysis of Sediments A, B, and C and Extract F[a]

	Sediment A	Sediment B	Sediment C	Extract F
Number of laboratories reporting results	33	35	35	35
Number of outliers[b]	0	1	0	0
Outliers laboratory number		29		
Mean	0.540	0.546	0.702	0.702
Mean exc. outliers	0.540	0.543	0.702	0.702
Median	0.552	0.546	0.704	0.704
Repeatability standard deviation, s_r	0.016	0.013	0.006	0.014
Repeatability relative standard deviation	3%	2%	1%	2%
Repeatability limit	0.046	0.036	0.017	0.039
Reproducibility standard deviation, s_R	0.053	0.030	0.023	0.027
Reproducibility relative standard deviation	10%	5%	3%	4%
Reproducibility limit	0.148	0.083	0.066	0.075

[a]Median, repeatability, and reproducibility are calculated after outlier removal.
[b]Outliers were determined from box plots of the data (i.e., data that fall beyond the whiskers).

0.028 (1.6°C) and 0.017 (1.0°C), respectively, with reproducibility of 0.067 (3.8°C) and 0.050 (2.8°C), respectively.

[22] To investigate potential causes for differences among laboratories, we plotted TEX$_{86}$ values of Sediment B against Sediment C (Figure 5a). The differences between TEX$_{86}$ measurements are consistent within individual laboratories, i.e., most laboratories tend to have all of their values either lower or higher than the mean. This suggests that the differences between laboratories are not caused by heterogeneity between individual vials of the

samples. Rather, it suggests that differences are caused by instrumental characteristics, as previously suggested [*Schouten et al.*, 2009].

[23] The results obtained for TEX$_{86}$ analysis thus compare well to those obtained in the previous round-robin study, particularly in light of the fact that most results are now obtained for sediments rather than extracts. Intralaboratory precision (repeatability) has improved to <1°C. This suggests that improvements have been made in internal lab consistency over the years, possibly by increased experience with HPLC-MS techniques
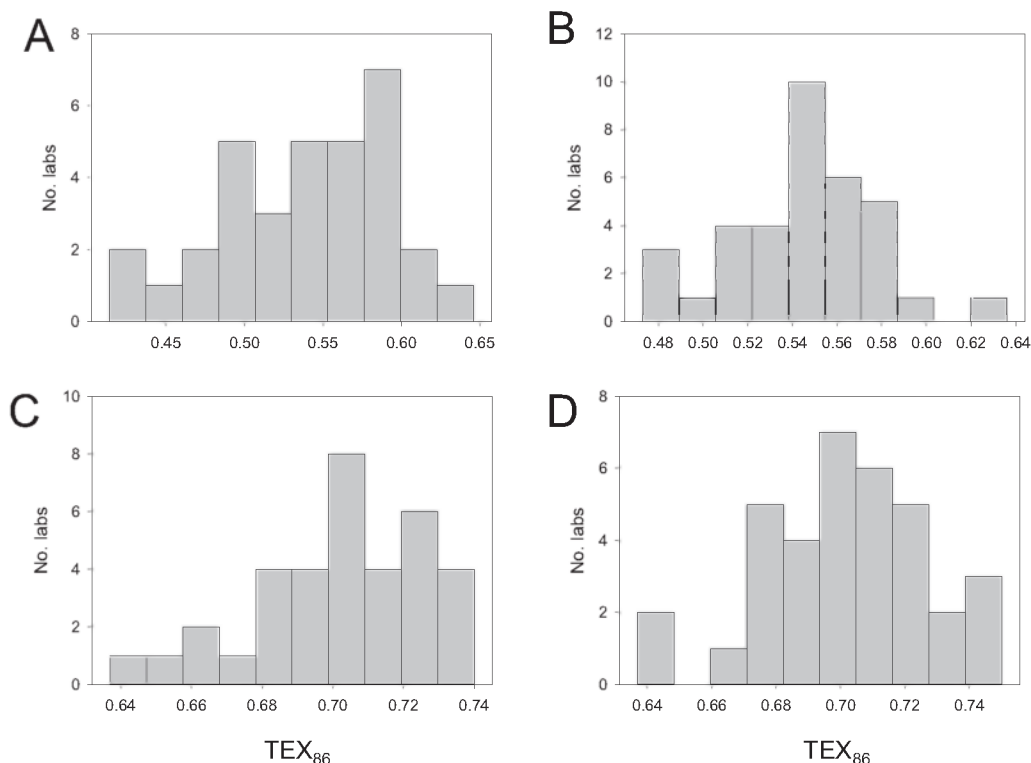

Figure 4. Histogram of TEX$_{86}$ values of (a) Sediment A, (b) Sediment B, (c) Sediment C, and (d) Extract F.
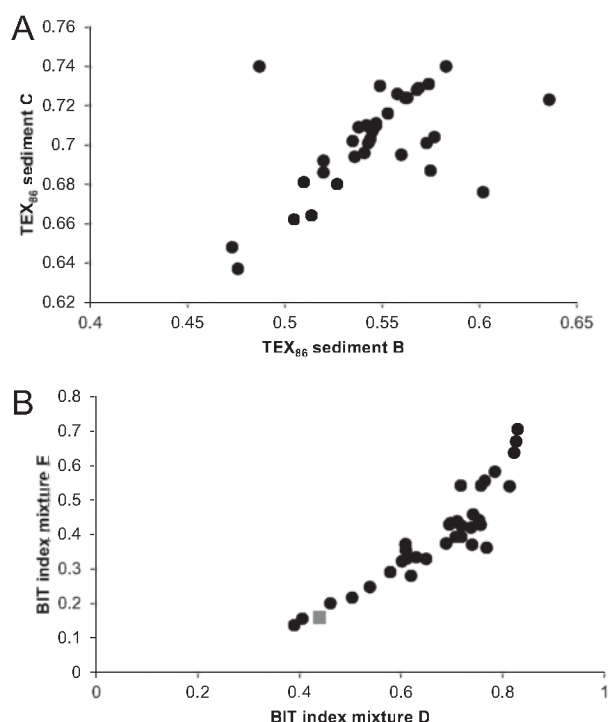
Figure 5. Crossplots of (a) TEX$_{86}$ of Sediment B against Sediment C and (b) BIT index for GDGT mixture. Red square indicates the molar BIT indices of the GDGT mixtures.

as speculated by *Schouten et al.* [2009] and/or improved MS systems with higher sensitivity. There is also some improvement in consistency between labs (i.e., the reproducibility improved) which, when translated into temperature, corresponds to reducing the variability from 3–4 to 1–3°C. The TEX$_{86}$ analysis now performs relatively well compared to round-robin studies of other paleothermometers. *Rosell-Melé et al.* [2001] found for U$_{37}^{k'}$ analyses of several sediments a repeatability of 1.6°C, slightly worse than our results, but their reproducibility of 2.1°C is similar or better than obtained in our study. *Rosenthal et al.* [2004] reported a repeatability of 1–2°C and a reproducibility of 2–3°C for Mg/Ca analysis of foraminifera, numbers which are similar to this study. Hence, it seems that TEX$_{86}$ measurements in the different geochemical labs have become comparable in robustness and consistency to those of other temperature proxy measurements.

3.2. BIT Analysis of Sediments and Extract

[24] The results of the analysis of the BIT index are displayed in Tables 4 and 6 and Figures 3, 6, and 7b. Sediment C is an open marine setting with

a small contribution of soil OM, and thus, values are nearly all below 0.1 (Figures 3c and 6c, Table 4) with a mean value, after outlier removal, of 0.027 (Table 6). Extract F, the extract of Sediment C, had a similar mean BIT value of 0.037 (Figures 3f and 6f and Tables 4 and 6). Sediment A is from a lake (Salt Pond, MA, USA) that likely contains substantial amounts of soil organic carbon. Indeed, higher BIT indices were measured for this sediment than for Sediment C (Figures 3a and 6a, Table 4) with a mean value of 0.951 (Table 6). Sediment B is from a coastal shelf (Carolina Margin), which in principle can contain a range of soil OM input [*Schouten et al.*, 2013, and references cited therein]. The BIT values were consistently low (Figures 3b and 6c, Table 4) with a mean value, after outlier removal, of 0.096 (Table 6), suggesting relatively little input of soil OM in this region.

[25] The three sediments and the extract thus have fairly extreme values, close to 0 (Sediments B and C and Extract F; Figure 7b) or close to 1 (Sediment A; Figure 7b). This has consequences for the repeatability and reproducibility, as numbers close to the extreme end of the indices will artificially have a better repeatability and reproducibility. Indeed, repeatability varied between 0.002 and 0.017 and reproducibility varied between 0.013 and 0.042, slightly better than for TEX$_{86}$ analysis (Tables 5 and 6). The results are similar to the Arabian Sea extract analyzed in the previous round-robin study, which also had a low BIT index value, but much better than the repeatability and reproducibility of the Drammensfjord extract which had an intermediate BIT index value of 0.588 (mean of measurements) [*Schouten et al.*, 2009]. Indeed, the two mixtures of isolated GDGTs, which had intermediate BIT indices, show a much larger repeatability and reproducibility, as their values are not close to the extremes (see 3.4). Nevertheless, the results do suggest that extreme values of BIT indices are fairly consistently measured between laboratories (Figure 7b), in contrast to intermediate values between ~0.2 and ~0.8, suggesting at least sediments with relatively "low" or "high" soil OM input can be distinguished.

3.3. Impact of Sample Work Up and Mass Spectrometry Techniques

[26] The inclusion of both sediment and its extract (Sediment C and Extract F, respectively) in the round-robin analysis allowed the impact of
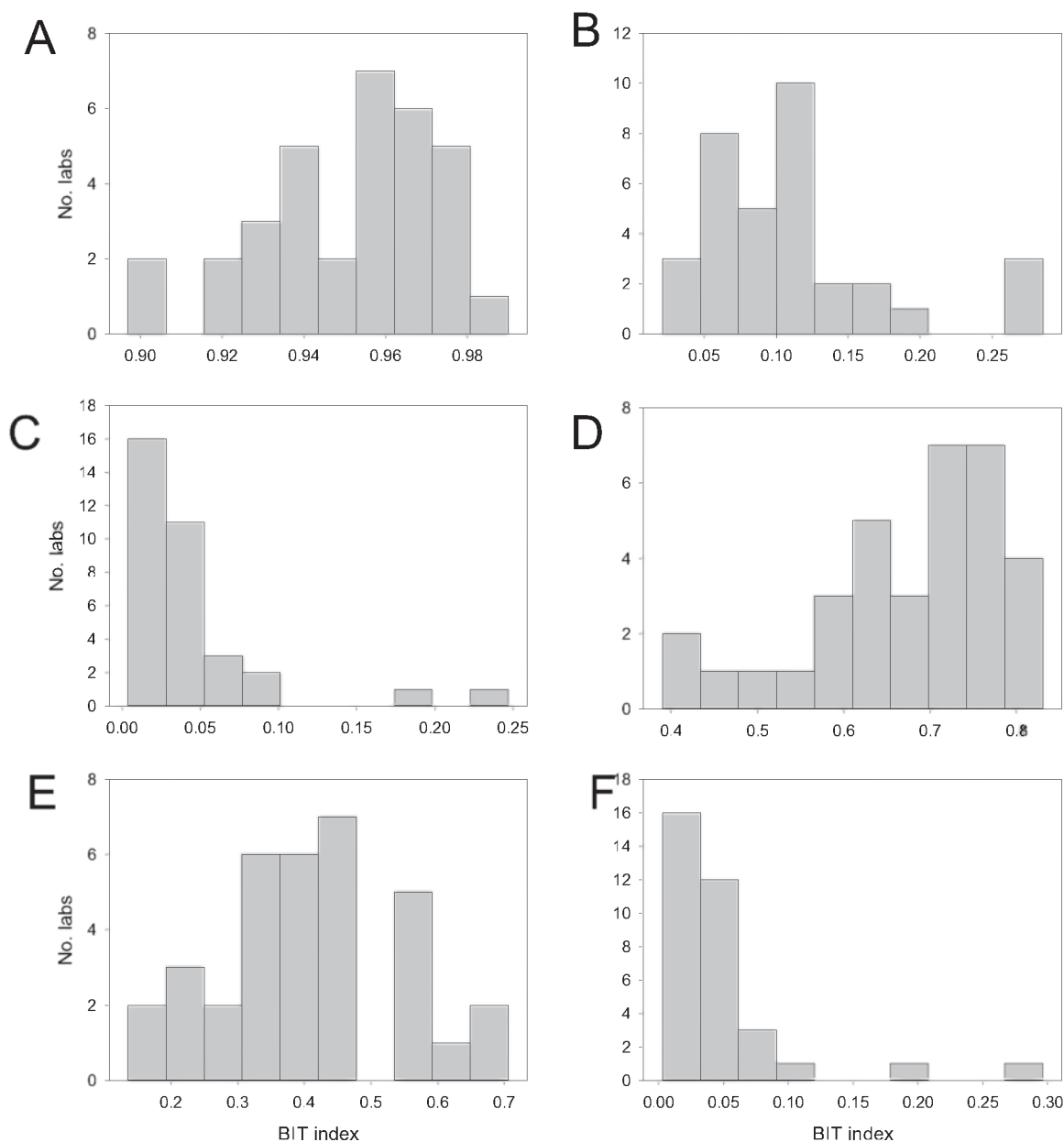
Figure 6. Histogram of BIT index values of (a) Sediment A, (b) Sediment B, (c) Sediment C, (d) GDGT Mixture D, (e) GDGT Mixture E, and (f) Extract F.

extraction methods to be evaluated. Several different extraction techniques were used including ultrasonic, microwave, accelerated solvent and Soxhlet extraction (Table 1). Most of the solvents used consisted of mixtures of DCM and MeOH, although in some cases a "Bligh & Dyer" type extraction, using a buffer [*Bligh and Dyer*, 1959], was used. The extracts were also processed in different ways including no treatment, hydrolysis, or column separations using SiO_2 or Al_2O_3 (Table 1). A first evaluation can be made by comparing the mean and median BIT and TEX$_{86}$ values of Sedi-

ment C and Extract F. This showed that both TEX$_{86}$ (0.702 versus 0.702) and BIT values (0.027 versus 0.037) are nearly identical and well within the repeatability limits (Tables 5 and 6). Thus, on a general level the impact of sample processing is relatively small, although it should be noted that a substantial number of the participants used a similar workup for the Sediment C as was used for preparing Extract F. Comparison on the individual laboratory level between TEX$_{86}$ values obtained from Sediment C and Extract F shows differences varying from −0.019 to 0.021 corresponding to

Table 6. Summary Statistics of BIT Analysis of Sediments A, B, and C, the GDGT Mixtures D and E, and Extract F[a]

	Sediment A	Sediment B	Sediment C	Mixture D	Mixture E	Extract F
Number of laboratories	33	34	34	34	34	35
Number of outliers[b]	0	3	4	2	1	2
Outliers I.D.		1, 2, 29	2, 16, 21, 29	3, 7	2	2, 29
Mean	0.951	0.114	0.043	0.676	0.403	0.047
Mean exc. Outliers	0.951	0.096	0.027	0.690	0.384	0.037
Median	0.957	0.110	0.031	0.710	0.393	0.034
Repeatability standard deviation, s_r	0.007	0.011	0.002	0.017	0.009	0.004
Repeatability relative standard deviation	1%	11%	7%	3%	2%	11%
Repeatability limit	0.020	0.030	0.005	0.048	0.026	0.011
Reproducibility standard deviation, s_R	0.022	0.042	0.013	0.107	0.139	0.024
Reproducibility relative standard deviation	2%	43%	49%	15%	36%	66%
Reproducibility limit	0.062	0.177	0.038	0.299	0.390	0.068

[a]Median, repeatability, and reproducibility are calculated after outlier removal.
[b]Outliers were determined from box plots of the data (i.e., data that fall beyond the whiskers).

−0.8 to 0.9°C when converted to temperature using *Kim et al.* [2008]. Differences in BIT values vary only between −0.013 and 0.049. These differences are all relatively minor, suggesting that the type of extraction method and extract processing do not have a large impact on GDGT distributions, in agreement with previous observations [*Schouten et al.*, 2007; *Escala et al.*, 2009; *Lengger et al.*, 2012].

[27] The different types of mass spectrometers used allow us to assess potential differences between mass spectrometry techniques (Table S2). Comparison of the TEX$_{86}$ and BIT measurements of triple quadrupole mass spectrometers with those of single quadrupole mass spectrometers, the most commonly used technique, shows no significant differences (Student's t test, $p > 0.05$). This is perhaps not surprising as the triple quadrupole mass spectrometers were used in single quadrupole mode. However, comparison of the results of ion trap mass spectrometers with those of single quadrupole mass spectrometers shows significant differences (Student's t test, $p < 0.05$), i.e., slightly lower values for TEX$_{86}$ values of Sediment C (0.684 versus 0.713) and Extract F (0.684 versus 0.710) and substantially lower BIT values for standard Mixtures D (0.558 versus 0.711) and E (0.295 versus 0.445). This may suggest that ion trap mass spectrometers, in general, yield slightly lower TEX$_{86}$ values but especially lower BIT values.

3.4. Comparison of MS-Based BIT Index and Mass-Based BIT Index

[28] Until now the BIT index, as well as the TEX$_{86}$, has been an empirical ratio solely based on MS response. The last round-robin exercise dem-

onstrated that this approach had especially large consequences for the BIT index as the extract analyzed with intermediate BIT value (mean 0.588)
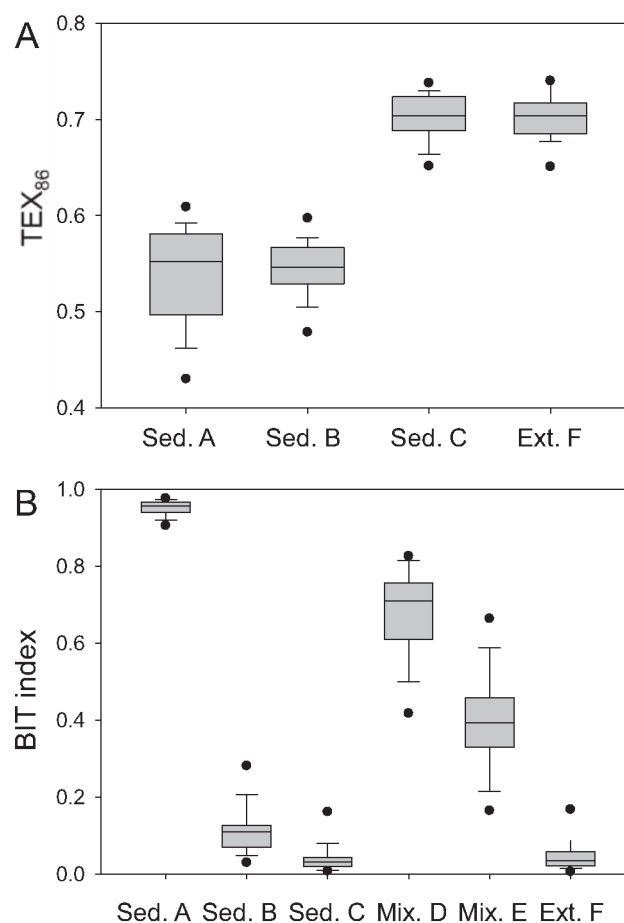

Figure 7. Box plots of (a) TEX$_{86}$ values of Sediments A, B, and C and Extract F and (b) BIT index values of Sediments A, B, and C, GDGT Mixtures D and E, and Extract F. The horizontal line within the box represents the median sample value. Box indicates lower 25% and upper 75% percentiles and bars indicate lower 10% and upper 90% percentiles.
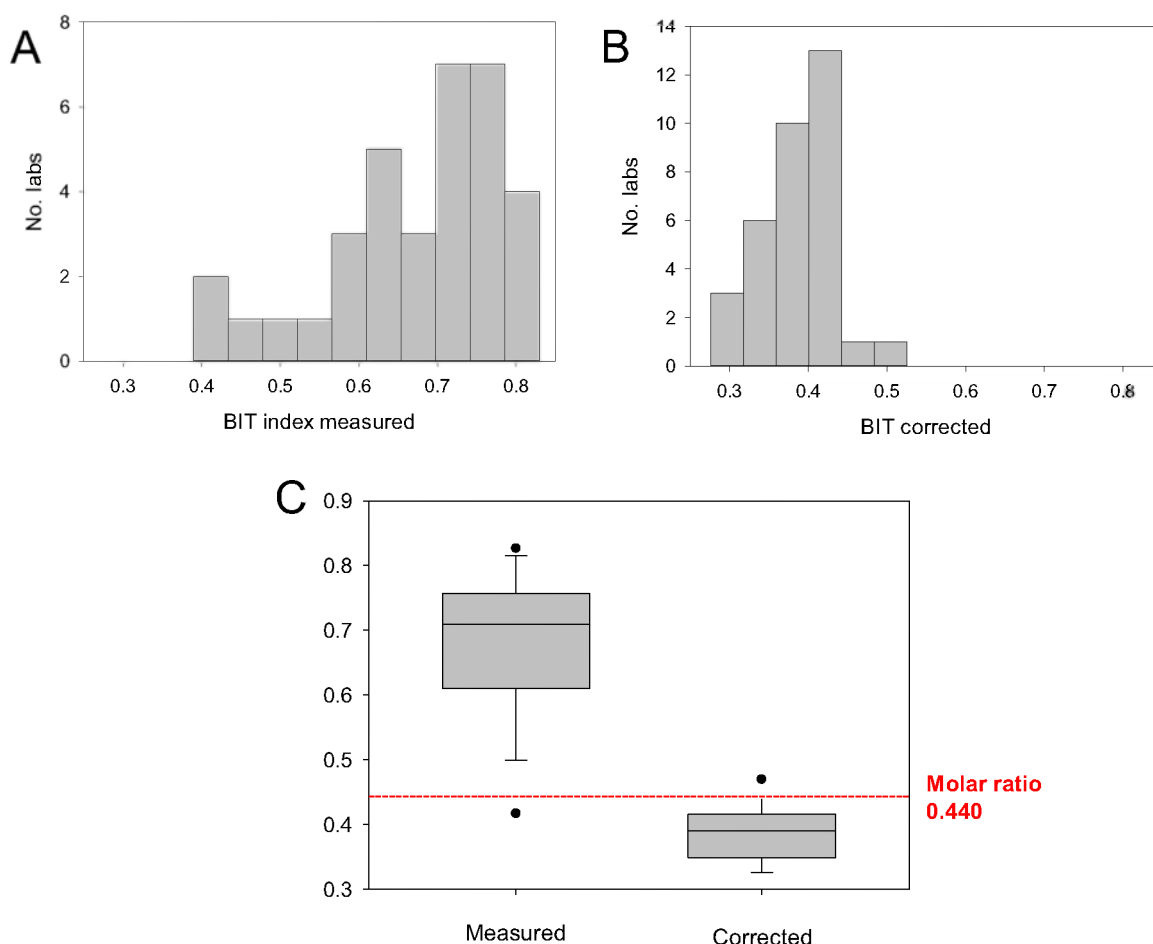
Figure 8. (a, b) Histograms and (c) box plots of BIT index values of GDGT Mixture D and corrected BIT index values, based on correction factors obtained from measurements of Mixture E. Box indicates lower 25% and upper 75% percentiles and bars indicate lower 10% and upper 90% percentiles. The dotted red line is the BIT index based on the molar ratios of GDGTs in Mixture D. The horizontal line within the box represents the median sample value.

had a very large spread in values (from 0.340 to 0.821) due to the different MS instrument responses [cf. *Escala et al.*, 2009]. The use of mixtures of GDGT standards in the current round-robin analysis allows, for the first time, a comparison between BIT values measured by the HPLC-MS (BIT$_{MS}$) with those based on mass (BIT$_{mass}$) or moles (BIT$_{mol}$) of GDGTs. Two GDGT mixtures were prepared with different ratios between crenarchaeol versus branched GDGT-I and GDGT-II (i.e., a BIT$_{mol}$ of 0.440: Mixture D and 0.158: Mixture E), respectively. As expected, highly variable BIT$_{MS}$ values ranging from 0.390 to 0.830 for Mixture D and 0.136 to 0.706 for Mixture E, respectively, were reported (Figures 3d and 3e and Table 4). A broad range of nonuniform distributions was found (Figures 6d and 6e), similar to the previous round-robin results, leading to poor

reproducibilities for BIT measurements of the GDGT mixtures (Table 6). Comparison of the BIT$_{MS}$ values of each laboratory showed that the trends were consistent between laboratories, i.e., laboratories producing high BIT$_{MS}$ values of Mixture D also produced high BIT$_{MS}$ values for Mixture E and vice versa (Figure 5). Interestingly, nearly all BIT$_{MS}$ values reported were higher than the BIT$_{mol}$ of the GDGT mixtures (Figure 5b). Only laboratories 3, 7, and 18 obtained BIT$_{MS}$ values similar (i.e., within 0.05) to that of the BIT$_{mol}$ of the GDGT mixtures. This suggests that BIT values previously reported in the literature were nearly all overestimating the "true" molar-based BIT index. The cause of the overestimation is likely due to a higher response factor of branched GDGTs compared to crenarchaeol in most of the MS systems used. The three laboratories that have

Table 7. BIT$_{MS}$ Values of Mixture E, the Calculated Correction Factor for Differences in MS Response of Branched GDGTs and Crenarchaeol, the BIT$_{MS}$ Values of Mixture D, and Corrected BIT$_{mol}$ Values of Mixture D After Applications of Equations (5) and (6)[a]

Laboratory Number	BIT$_{MS}$ Mixture E	Correction factor	BIT$_{MS}$ Mixture D	SD	n	Estimated BIT$_{mol}$ Mixture D
1	0.670	10.8	0.827	0.012	7	0.307
2	0.706	12.8	0.830	0.000	5	0.276
3	0.136	0.84	0.390	0.023	6	0.433
4	0.458	4.50	0.742	0.004	5	0.390
5	0.438	4.15	0.711	0.022	3	0.372
6	0.374	3.18	0.689	0.002	5	0.411
7	0.155	0.98	0.406	0.006	5	0.412
8	0.354	2.92	0.610	0.036	5	0.349
9	0.330	2.62	0.612	0.003	3	0.376
10	0.280	2.07	0.620	0.000	3	0.441
11	0.322	2.53	0.602	0.008	5	0.374
12	0.441	4.20	0.754	0.005	5	0.422
13	n.r.		n.r.			
14	0.429	4.00	0.695	0.005	4	0.363
15	0.556	6.66	0.765	0.013	3	0.328
16	0.582	7.41	0.785	0.002	3	0.330
17	0.428	3.98	0.757	0.001	3	0.439
18	0.200	1.33	0.461	0.004	6	0.391
19	0.329	2.61	0.650	0.003	3	0.416
20	0.370	3.12	0.740	0.104	3	0.477
21	0.540	6.25	0.814	0.011	5	0.412
22	0.433	4.06	0.698	0.008	6	0.363
23	0.425	3.93	0.719	0.006	3	0.394
24	0.542	6.3	0.758	0.004	5	0.332
25	0.216	1.47	0.504	0.005	5	0.409
26	0.420	3.85	0.738	0.008	5	0.422
27	0.247	1.75	0.539	0.005	5	0.401
28	0.372	3.15	0.609	0.001	3	0.331
29	0.637	9.34	0.823	0.006	3	0.332
30	0.393	3.44	0.708	0.001	5	0.413
31	0.334	2.67	0.630	0.000	5	0.390
32	0.393	3.44	0.719	0.001	3	0.426
33	0.542	6.30	0.718	0.003	5	0.288
34	0.361	3.01	0.769	0.009	5	0.526
35	0.291	2.18	0.579	0.001	5	0.386

[a]n.r., not reported.

BIT$_{MS}$ values close to BIT$_{mol}$ values all use a Bruker-manufactured MS, suggesting that certain MS systems may suffer less from this differential MS response. In any case, the results highlight the need to use GDGT standards to properly estimate BIT$_{mol}$ values or even concentrations of branched GDGTs [cf. *Huguet et al.*, 2006].

[29] The overestimation of BIT values may have several implications. For example, it likely means that past efforts to estimate the relative amount of soil organic carbon in marine sediments based on the BIT index [e.g., *Weijers et al.*, 2009; *Belicka and Harvey*, 2009; *Kim et al.*, 2010b] may have overestimated the contribution of soil organic carbon. However, this error is likely minor compared to the assumption that concentrations of branched GDGTs in soil organic carbon and crenarchaeol in marine organic carbon are similar and do not vary over time [cf. *Weijers et al.*, 2009]. Nevertheless, the use of GDGT standards will likely lead to a better assessment of the relative contribution of soil organic carbon in the marine environment. Another point is that BIT values >0.3 are used to indicate potential biases in TEX$_{86}$ due to input of soil-derived isoprenoid GDGTs [*Weijers et al.*, 2006]. However, as discussed in *Schouten et al.* [2013], the recommended cutoff value heavily depends on the "terrestrial TEX$_{86}$" value as well as the concentrations of isoprenoid versus branched GDGTs, which will likely vary on a regional scale. Thus, TEX$_{86}$ might be biased at BIT values <0.3 or not biased at BIT values >0.3. In our previous round-robin study [*Schouten et al.*, 2009] we suggested to correlate BIT values with TEX$_{86}$ values and in case of significant correlations use this as a red flag. Our results here do not change this recommendation.

[30] The use of two GDGT mixtures also allows the differences in relative response of crenarchaeol versus branched GDGTs to be assessed for each MS. These specific response factors can be used to correct BIT$_{MS}$ values to BIT$_{mol}$ values. We first calculated a correction factor, F_{corr}, for the differences in MS response for crenarchaeol versus branched GDGTs for each MS system based on the results of GDGT Mixture E:

$$Fcorr = \frac{C/B}{\frac{1}{BIT_{MS}} - 1} \quad (5)$$

in which C/B is the molar ratio of crenarchaeol versus branched GDGTs of standard Mixture E, i.e., 5.32 (0.78 µmol of crenarchaeol versus 0.097 µmol of GDGT-II and 0.049 µmol of GDGT-I). This correction factor can then be applied to the reported BIT$_{MS}$ values of Mixture D to estimate BIT$_{mol}$ values for this mixture:

$$Estimated\ BITmol = \frac{1}{\frac{Fcorr}{BIT_{MS}} - Fcorr + 1} \quad (6)$$

[31] The calculations show that corrected BIT$_{mol}$ values are in a much smaller range than the reported BIT$_{MS}$ values and have a more unimodal distribution (Figures 8a and 8b and Table 7). Reproducibility also substantially improves from 0.107 to 0.059, and the mean and median values of the estimated BIT$_{mol}$ values, 0.386 and 0.391, are now much closer to the actual BIT$_{mol}$ value of 0.440 (Figure 8c). This shows that the use of GDGT standard mixtures can substantially improve interlaboratory consistency of the BIT index and leads to better estimates of BIT$_{mol}$ values.

4. Conclusions

[32] Our extensive round-robin study of TEX$_{86}$ and BIT analyses, involving 35 laboratories and using 3 sediments, 1 sediment extract, and 2 GDGT mixtures, showed that measurements of the TEX$_{86}$ and BIT index were improved compared to the previous round-robin study, i.e., an improved intralaboratory precision (repeatability) as well as improved consistency (reproducibility) between labs. Importantly, comparison of the results obtained from one sediment and its extract showed that TEX$_{86}$ and BIT index are not affected substantially by sediment extraction and processing techniques. Comparison of measured BIT values with those of two GDGT mixtures with known ratios of crenarchaeol and branched GDGTs

showed that measured BIT values generally overestimate the BIT index based on the molar ratios of the GDGTs. A correction for this different mass spectrometric response based on the GDGT mixture showed a considerable improvement in the reproducibility of BIT index measurements between laboratories, suggesting that standard mixtures should be used in order to obtain consistent BIT values as well as molar-based BIT values.

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