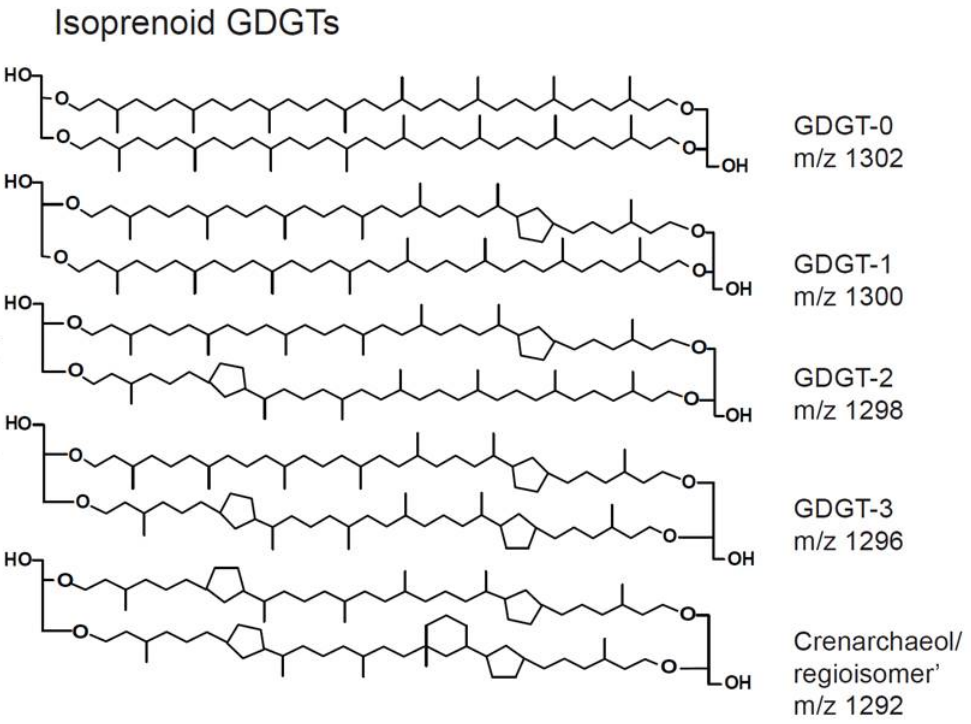
Supplementary figure legends

Fig. S1. Structures of isoprenoid core-GDGTs discussed in the text (left) and the most commonly detected IPL types (right).

Fig. S2. HPLC-ESI/MS Extracted ion current (EIC) of m/z 1643.4549 within 3 ppm mass accuracy representing the ammoniated molecule of DH-GDGT-0 in subsurface sediment (10-12 cm) at 1306 mbsl. The chromatogram shows three separate peaks representing three different isomers (the peak at 19.39 min is the signal of the second isotopologue of GDGT-1). MS^2 mass spectra of each of the three isomers are shown in panel a-c in order of retention time. Panel a shows a single fragment ion at *m/z* 1302. Panel b shows an additional fragment ion at m/z 1284. Panel c shows a main fragmentation product at *m/z* 1464. The fragmentation product at *m/z* 1302 shown in panel a represents the core lipid of GDGT-0 resulting from the loss of 2 hexose moieties from DH-GDGT-0, with 1 hexose moiety attached to either side of the molecule. The additional fragment ion at *m/z* 1284 shown in panel b represents the loss of an additional water from the GDGT-0 core lipid, which can only occur if a free alcohol moiety is present and thus both hexose moieties are postulated to be attached to one side of the GDGT-0 molecule. Panel C shows the loss of a single hexose from the *m/z* 1643 parent, but no further fragmentation. suggesting that this isomer is in fact the IPL-type HCP GDGT, where the hexose is positioned at the opposite side from the cyclopentanetetraol moiety. If the hexose moiety would have been bound to the cyclopentanetetraol moiety, as suggested by Sturt et al., 2004, an additional loss of water from the MH-GDGT-0 would have been expected, similar to what was observed in the fragmentation of DH-GDGT-0 shown in panel b.

Supplementary figures and tables

Fig. S1.



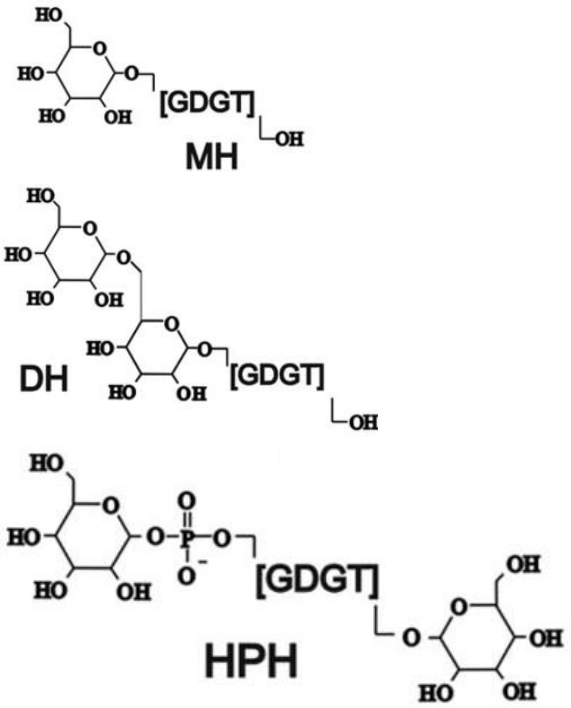
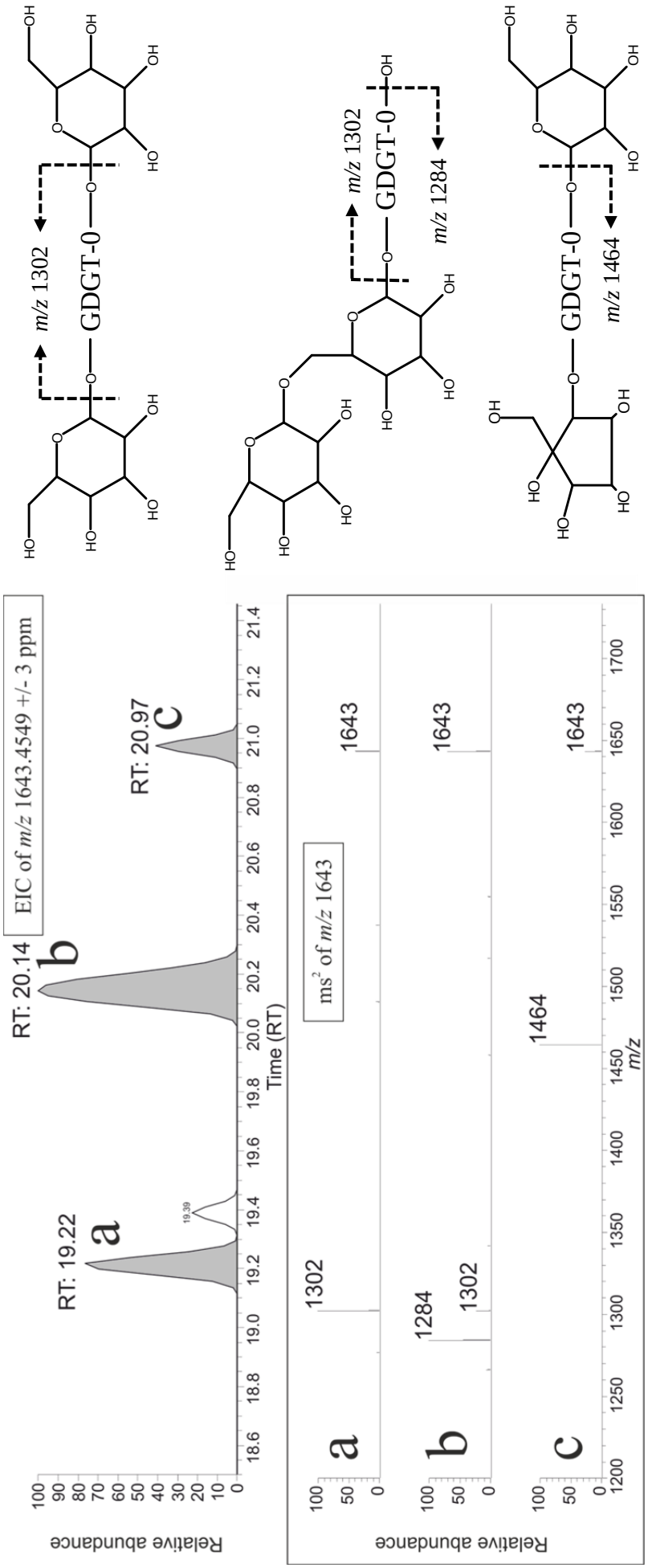


Table S1. PCR primers, efficiency and R^2 of the QPCR assays

QPCR (gene)	Primers	Ref	Efficiency	R^2
16S rRNA	Parch519F/ARC915R	Pitcher et al., 2011	99.8	0.96
16S rRNA	Parch519F/ARC915R	Pitcher et al., 2011	87.5	1.00
16S rRNA	Parch519F/ARC915R	Pitcher et al., 2011	90.0	0.99
*Amo*A	CrenAmoAQ-F/CrenAmoAModR	Pitcher et al., 2011	97.5	1.00
*Amo*A	CrenAmoAQ-F/CrenAmoAModR	Pitcher et al., 2011	78.5	1.00

Fig. S2.


m/z 1302
GDGT-0
m/z 1302
GDGT-0
m/z 1284
GDGT-0
m/z 1464
EIC of m/z 1643.4549 +/- 3 ppm
RT: 19.22
a
19.30
RT: 20.14
b
RT: 20.97
c
Relative abundance
Time (RT)
ms2 of m/z 1643
1302
1284
1302
1464
1643
1643
1643
m/z