

1 Dear editor,

2

3 We hereby submit the revised version of our manuscript (bg-2014-375), now entitled  
4 'A laboratory experiment on the behavior of soil-derived core and intact polar GDGTs  
5 in aquatic environments'.

6

7 We have followed most of the suggestions of the three anonymous referees, as you  
8 can read in the replies posted online. Below we list the major changes that we have  
9 made to the manuscript. All these changes are highlighted in the hereby submitted  
10 version. We hope that you find this revised version suitable for publication in  
11 Biogeosciences.

12

13 On behalf of all co-authors,

14 Yours sincerely,

15 Francien Peterse

16

17

18 - Following the suggestion of referee #1 we have changed the title of our  
19 manuscript to better fit the scope and findings of the paper.

20 - We have added a map indicating the sample locations on request of referee  
21 #1 (Figure 2).

22 - Based on comments by referees #2 and #3, we have analyzed the TOC and TN  
23 concentrations in the incubated soils to provide more background data to  
24 improve the interpretation of our incubation results. We have added TOC and  
25 TN sections to the methods (2.2) as well as the results&discussion (3.1).  
26 These data are also depicted in Figure 3.

27 - We have added GDGT-release from the soil matrix as an alternative  
28 explanation for the increase in (iso)GDGT concentrations over the course of  
29 the experiment, as suggested by referee #2.

30 - Following the suggestions of referees #2 and #3 we have clarified our use of  
31 0.7 GF/F and 0.2 PES membrane filters to obtain SPM samples and also  
32 included the references to Ingalls et al., 2012 and Close et al., 2014.

33 - We have added r2 and p-values to the manuscript where appropriate.

34 - We have generally toned down our discussion on the presumed  
35 lability/stability/production/degradation of IPL-GDGTs, as all referees pointed  
36 out correctly that identification of the type of headgroup was needed to  
37 support our interpretations, and have added the suggested additional  
38 references.

39 - We have added the reference to Lincoln et al., 2014, as suggested by referees  
40 #2 and #3.

41 - Based on comments of all referees on i) the duration of the experiment, ii)  
42 the design of the control setup, iii) the soil:water ratio used, we have added a  
43 section to the discussion in which we evaluate our experimental setup and  
44 make suggestions for future experiments based on a.o. aforementioned main  
45 concerns. This section has replaced the section in the original manuscript in  
46 which GDGT distributions in soil and river SPM were discussed. This issue is  
47 now also addressed in the evaluation section.

48

# A laboratory experiment on the behavior of soil-derived core and intact polar GDGTs in aquatic environments

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## Abstract

We have performed incubation experiments in order to examine the behavior of soil-derived branched glycerol dialkyl glycerol tetraether (brGDGT) membrane lipids upon entering an aquatic environment and to evaluate the processes that potentially take place during their fluvial transport from land to sea. We incubated a soil from the Rakaia River catchment on the South Island of New Zealand using Rakaia River water and ocean water collected near the river mouth as inocula for a period of up to 152 days. The concentrations, as well as the relative distribution of brGDGTs derived from intact polar ('living'; IPL) lipids and core ('fossil'; CL) lipids remained unaltered over the course of the experiment. In contrast, the total pool of isoprenoid GDGTs (isoGDGTs), currently attributed to soil *Thaumachaeota*, increased substantially (27-72%) in all incubation setups by the end of the experiment. As a consequence, a decrease in Branched and Isoprenoid Tetraether (BIT) index values - a proxy for the relative input of fluvially discharged soil material into a marine system - became evident after an incubation period of 30 days, with a maximum final decrease of 0.88 to 0.74 in the experiment with river water. The relative distribution within the isoGDGT pool shows changes with time, suggesting that isoGDGT producers may either have different rates of membrane adaptation or production/degradation. Also preferential release from the soil matrix or a shift in source organism(s) may take

1 place. While the apparent stability of soil brGDGTs during this incubation experiment  
2 reinforces their potential as tracers for land-sea transport of soil organic carbon and  
3 their use in paleoclimate reconstructions, the distributional differences between  
4 GDGTs in river water and nearby soil, as well as in river and ocean water, indicate  
5 that further research is needed to pinpoint the sources of GDGTs that are ultimately  
6 discharged to the oceans and are subsequently archived in continental margin  
7 sediments.

8

## 9 **1 Introduction**

10 The global carbon cycle encompasses a myriad of biogeochemical processes that  
11 influence our climate and link all carbon reservoirs on the Earth surface. Soils are  
12 considered to play a very active and fundamental role in this cycle, as their  
13 mobilization from land and subsequent deposition in marine sediments serves as a  
14 long-term sink of atmospheric CO<sub>2</sub>. However, the exact magnitude and mechanisms  
15 of soil organic carbon (OC) transfer from terrestrial source to marine sink remain  
16 elusive (e.g. Cole et al., 2007; Weyhenmeyer et al., 2012), which partly stems from  
17 the lack of a suitable tracer of the soil OC pool. Our current insights in land-sea soil  
18 OC transport dynamics are primarily based on bulk properties of river suspended  
19 particulate matter (SPM). However, next to soil OC, river SPM also comprises carbon  
20 derived from aquatic production and ‘fossil’ carbon from rock erosion, each of which  
21 influences the bulk properties of SPM (Blair et al., 2010).

22 Analytical advances in the field of organic biogeochemistry have yielded a growing  
23 number of powerful tools with the potential to exclusively target components of  
24 specific pools, including soil OC. In this context, branched glycerol dialkyl glycerol  
25 tetraethers (brGDGTs; Fig. 1) have been put forward as tracers for soil-derived OC in  
26 carbon transport studies (e.g. Hopmans et al., 2004; Kim et al., 2006). Since their  
27 discovery (Sinninghe Damsté et al., 2000; Schouten et al., 2000), brGDGTs have been  
28 found in soils and peats worldwide (Weijers et al., 2007b, 2009; Liu et al., 2010). The  
29 exact organism(s) that produce(s) these compounds have yet to be identified, but  
30 current evidence points towards an origin from heterotrophic soil bacteria (Pancost  
31 and Sinninghe Damsté, 2003; Oppermann et al., 2010; Weijers et al., 2010) from the  
32 phylum of *Acidobacteria* (Weijers et al., 2009; Peterse et al., 2010; Sinninghe Damsté  
33 et al., 2011). BrGDGTs have been used as a proxy for the relative input of fluvially

1 transported soil OC in marine systems based on their abundance in coastal marine  
2 sediments relative to that of crenarchaeol (Fig. 1), an isoprenoid GDGT (isoGDGTs)  
3 produced by marine *Thaumarchaeota* (Sinninghe Damsté et al., 2002), parameterized  
4 as the Branched and Isoprenoid Tetraether (BIT) index (Hopmans et al., 2004).  
5 Moreover, subtle variations in the molecular distribution of the brGDGTs have been  
6 found to relate to mean annual air temperature and soil pH (Weijers et al., 2007b),  
7 indicating their potential for utility in paleoclimate studies. Indeed, using a  
8 combination of the Methylation of Branched Tetraethers (MBT) and Cyclisation of  
9 Branched Tetraether (CBT) indices (the so-called “MBT-CBT” proxy; Weijers et al.,  
10 2007b, recently revised as MBT<sup>2</sup>-CBT by Peterse et al., 2012), down-core variations  
11 in brGDGT distribution in fluvially-dominated continental margin sediments have  
12 been used to obtain an integrated climate history of the adjacent drainage basin (e.g.  
13 Weijers et al., 2007a).

14 Although GDGT-based proxies are finding increase use, our understanding of  
15 mobilization and transfer of soil OC and associated GDGT signals to fluvial  
16 environments, and the processes acting upon these components during transport are  
17 still poorly understood. There is emerging evidence, for example, for *in situ*  
18 production of brGDGTs in aquatic environments including high-latitude fjord systems  
19 (Peterse et al., 2009), open shelf sediments (Zhu et al., 2011), lakes (e.g. Sinninghe  
20 Damsté et al., 2009; Tierney and Russell, 2009), and most recently, rivers (De Jonge  
21 et al., 2014; Kim et al., 2012; Zell et al., 2013b; Zhang et al., 2012). The presence of  
22 intact polar lipid (IPL) precursors of brGDGTs in SPM from the Amazon (Zell et al.,  
23 2013b), Pearl (Zhang et al., 2012), and Yenisei rivers (De Jonge et al., 2014) provides  
24 strong evidence for aquatic brGDGT production, as the IPL head groups are thought  
25 to be rapidly lost upon cell death (Harvey et al., 1986; White et al., 1979), **although**  
26 **recent studies suggest that IPLs with ether bound headgroups may actually have very**  
27 **slow turnover rates in marine sediments (e.g. Logemann et al., 2011; Xie et al., 2013).**  
28 Regardless, the core lipids (CLs) that are released after IPL degradation are  
29 considered to represent ‘dead’, or fossil material - the fraction that is considered to be  
30 stored in sedimentary archives - and targeted for paleoclimate reconstruction. Hence,  
31 during fluvial transport from land to sea, the initial soil-derived brGDGT distribution  
32 entering a river may be modified by the addition of aquatic produced brGDGTs with a



1 potentially different signature, but also by microbial degradation and transformation  
2 of the soil brGDGTs.

3 In order to explore the latter process, i.e. the behavior of soil brGDGTs upon entering  
4 an aquatic system, we performed a laboratory experiment in which we incubated soil  
5 using river water or ocean water from near the mouth of the same river as microbial  
6 inocula. In the frame of a larger study on fluvial transport of soil organic carbon from  
7 land to sea on the South Island of New Zealand, we used soil and river water from the  
8 Rakaia River catchment for the experiment. In addition, the braided character of the  
9 Rakaia River may reduce the potential contribution of in situ produced brGDGTs due  
10 to the generally harsh and unstable conditions in such river systems. This should  
11 enable the monitoring in a controlled setting of what could also be an actual soil-  
12 derived brGDGT signal in the river. For that matter, concentration and distributional  
13 changes in IPL-derived and CL-brGDGTs were determined at different time intervals  
14 during the incubation experiment. Although isoGDGTs (Fig. 1) are primarily  
15 associated with marine archaea, they are also produced by soil *Thaumarchaeota*  
16 (Sinninghe Damsté et al., 2012), albeit in small amounts relative to the brGDGTs  
17 (Weijers et al., 2006b). In the marine realm, changes in isoGDGT distributions form  
18 the basis for the TEX<sub>86</sub> index, which is used as a proxy for sea surface temperature  
19 (SST; Schouten et al., 2002; Kim et al., 2010). Large contributions from soil  
20 isoGDGTs to the total isoGDGTs pool in marine sediments may consequently  
21 complicate the interpretation of TEX<sub>86</sub>-derived SST records from near continental  
22 margins (Weijers et al., 2006b). Therefore, all soil-derived GDGTs, i.e. both branched  
23 and isoprenoidal, were monitored during the experiment.

24

## 25 **2 Materials and methods**

### 26 **2.1 Sample collection and incubation setup**

27 The Rakaia River is one of the largest braided rivers on the South Island of New  
28 Zealand and has a mean annual discharge of 203 m<sup>3</sup>/s. The Rakaia is estimated to  
29 contribute 4.15 Mt of sediment a year, accounting for approximately 5% of the total  
30 South Island sediment yield to the adjacent continental shelf (Hicks et al., 2011). The  
31 river originates in the Southern Alps and flows through the Canterbury Plains before  
32 reaching the Pacific Ocean approximately 150 km from its source. Fresh water from

1 the river was sampled in jerry cans close to the town Rakaia (S 43°44'57.9", E  
2 172°01'52.9"), about 20 km upstream from the river mouth and outside of the tidal  
3 influence. The ocean water used as inoculum was collected from the shoreline (S  
4 43°54'15.1", E 172°11'41.9"), close to the river mouth (Fig. 2). Based on pH  
5 measurements, this water (pH 7.6) was strongly fluviually influenced (pH 7.5). Surface  
6 soil (0-10 cm; pH 5.8) was collected on the Canterbury Plains within the Rakaia River  
7 catchment (S 43°52'00.8", E 172°09'10.8"). All soil material and incubation water  
8 was collected in January 2012 and directly transported to the University of Otago,  
9 where the incubation experiment was set up the next day. SPM was collected by  
10 filtration of river and ocean water (100L onto a 0.2 µm polyethersulfone membrane  
11 and 10L onto a pre-combusted 0.7 µm GF/F, respectively) in the field, after which the  
12 filters were stored frozen.

13 In the laboratory at the University of Otago, the soil was sieved over a 2 mm mesh to  
14 remove roots and homogenize the sample, after which two 10 g subsamples of the soil  
15 were directly frozen at -20°C for subsequent determination of initial GDGT  
16 composition. For the incubations, twenty-eight 250 ml bottles were filled with 10 g  
17 soil subsamples, after which 100 ml of river water or ocean water, untreated to  
18 preserve the natural microbial community, was added to twelve bottles each. Distilled  
19 water was added to the remaining four bottles to serve as control setup. The  
20 incubation bottles were loosely plugged with cotton wool, creating quasi-aerobic  
21 conditions, and placed on a shaker table from January to June 2012. Based on IPL  
22 degradation rates observed in previous studies (Harvey et al., 1986; Logemann et al.,  
23 2011), two bottles with river water and two bottles with ocean water were taken from  
24 the shaker table and immediately placed into a freezer (-20°C) at 1, 7, 14, 30, 91, and  
25 152 days after the start of the experiment. Duplicate control samples were frozen after  
26 91 and 152 days. At the end of the experiment all bottles were shipped on ice to ETH  
27 Zürich, Switzerland, where they were briefly thawed and the supernatant was pipetted  
28 off. The soils were then freeze dried, and stored frozen at -20°C until subsequent  
29 sample work-up and analysis.

## 30 **2.2 Total organic carbon and nitrogen analysis**

31 The freeze-dried, homogenized soils were weighed in silver capsules and fumigated  
32 with 1M HCl for three days and neutralized with NaOH for two days in a desiccator

1 to remove carbonates prior to total organic carbon (TOC) and total nitrogen (TN)  
2 analysis. The measurements were performed on a vario MICRO elemental analyzer at  
3 ETH Zürich.

### 4 **2.3 GDGT extraction and analysis**

5 Freeze-dried soil samples (3-4 g) were solvent-extracted three times using a Bligh and  
6 Dyer technique modified from Sturt et al. (2004). In short, a solvent mixture of  
7 MeOH:dichloromethane (DCM): phosphate buffer at pH 7.4 (2:1:0.8, v/v/v) was  
8 added to the soils and ultrasonically extracted for 10 min. The extract was collected  
9 each time after centrifuging at 1000 rpm for 5 mins. DCM and phosphate buffer were  
10 added to the combined extracts to a volume ratio of 1:1:0.9 to obtain phase separation.  
11 The DCM phase, containing the GDGTs, was collected after centrifuging, and the  
12 remaining solvent was rinsed twice with DCM. The combined DCM phases were  
13 dried under N<sub>2</sub> and passed over a silica column (deactivated with 1 weight% water) to  
14 separate CLs and IPLs according to (Pitcher et al., 2009), with the exception that  
15 hexane:ethyl acetate 1:1 (v/v) was used to elute the CLs. An aliquot of the IPL  
16 fraction was analyzed directly for CLs in order to assess potential carry over. The  
17 remainder of IPL fraction was dissolved in 6N HCl in MeOH and heated at 100°C for  
18 at least 3h to release IPL-bound CLs. A known amount of C<sub>46</sub> GDGT standard  
19 (Huguet et al., 2006) was added to all fractions prior to analysis.

20 The filters were freeze dried and extracted with a MARS Xpress microwave  
21 extraction system, using DCM:MeOH 9:1 (v/v). After centrifugation (5 min at 400  
22 rpm), the total lipid extract (TLE) was pipetted off and the residues were rinsed twice  
23 with DCM:MeOH 9:1 (v/v). The combined extracts were dried under N<sub>2</sub> with a  
24 known amount of C<sub>46</sub> GDGT standard, after which the TLEs were separated into an  
25 apolar and a polar (GDGT) fraction by passing them over a silica (1% water  
26 deactivated) column using hexane:DCM 9:1 (v/v) and DCM:MeOH 1:1 (v/v),  
27 respectively.

28 All GDGT-fractions were dissolved in hexane:isopropanol 99:1 (v/v), filtered over a  
29 0.45 µm PTFE filter, and analyzed using high performance liquid  
30 chromatography/atmospheric pressure chemical ionization – mass spectrometry  
31 (HPLC/APCI-MS) with an Agilent 1260 Infinity series LC/MS at ETH Zürich  
32 according to Schouten et al. (2007a). Separation of the GDGTs was achieved with a

1 Grace Prevail Cyano column (3 $\mu$ m, 150 $\times$ 2,1mm) after passing through a guard  
2 column of the same material (5 $\mu$ m, 7,5 $\times$ 2,1mm) with hexane:isopropanol (99:1, v/v)  
3 as an eluent at a flow rate of 0.2 ml/min. The GDGT-fractions eluted isocratically  
4 with 90% A and 10% B for 5 min, and then with a linear gradient to 18%B for 34  
5 min, where A=hexane and B=hexane:isopropanol 9:1 (v/v). Selective ion monitoring  
6 of the [M+H]<sup>+</sup> was used to detect the different GDGTs. Although brGDGTs appear to  
7 have a higher response factor than isoGDGTs (Schouten et al., 2013a), quantification  
8 was done assuming similar response factors for all GDGTs and the internal standard.  
9 Hence, reported brGDGT concentrations are likely overestimated compared to those  
10 of the isoGDGTs. The amounts of all GDGTs are given as the average and the range  
11 of variation of the duplicate incubation samples. The amount of GDGTs in each  
12 sample has been corrected for carry over of CLs into the IPL fraction, which was on  
13 average <8% for brGDGTs and <1% for isoGDGTs.

#### 14 **2.4 GDGT-based index calculations**

15 The CBT and MBT' indices based on brGDGTs were calculated according to  
16 (Weijers et al., 2007b):

$$17 \text{ CBT} = -\log ((\text{Ib} + \text{IIb}) / (\text{Ia} + \text{IIa})) \quad (\text{Eq. 1})$$

18 and (Peterse et al., 2012):

$$19 \text{ MBT}' = (\text{Ia} + \text{Ib} + \text{Ic}) / (\text{Ia} + \text{Ib} + \text{Ic} + \text{IIa} + \text{IIb} + \text{IIc} + \text{IIIa}) \quad (\text{Eq. 2})$$

20 The BIT index was calculated following (Hopmans et al., 2004):

$$21 \text{ BIT} = (\text{Ia} + \text{IIa} + \text{IIIa}) / (\text{cren} + \text{Ia} + \text{IIa} + \text{IIIa}) \quad (\text{Eq. 3})$$

22 and for the TEX<sub>86</sub> index based on isoGDGTs the equation of (Schouten et al., 2002)  
23 was used:

$$24 \text{ TEX}_{86} = (\text{GDGT-2} + \text{GDGT-3} + \text{cren}') / (\text{GDGT-1} + \text{GDGT-2} + \text{GDGT-3} + \text{cren}') \quad (\text{Eq. 4})$$

26 Roman numerals and GDGT names refer to the molecular structures in Fig. 1.  
27 Regular reruns of selected samples on the HPLC-MS at ETH show that the analytical  
28 error on the indices is <0.01.

29

## 1 3 Results and discussion

### 2 3.1 TOC and TN concentrations over time

3 The TOC concentration in the soil is 1.6-1.7% at  $t=0$ , and varies in a range from 1.2-  
4 1.7%, whereas the TN concentration is 0.15% at  $t=0$  and remains practically stable  
5 (0.12-0.15) over the course of the experiment (Fig. 3). Given the limited range of  
6 variation in TOC and TN concentrations, no direct influence of TOC or TN on the  
7 GDGTs in this experiment is assumed during the following discussion.

### 8 3.2 BrGDGT concentrations over time

9 The total brGDGT pool (IPLs + CLs) initially present in the soil is  $239 \pm 13$  ng/g. In  
10 the different incubation experiments these concentrations varied between 187-325  
11 ng/g (river water), 186-322 ng/g (ocean water), and 252-407 ng/g (distilled water  
12 control) (Fig. 4). Although the abundance of CL-brGDGTs seems to increase with  
13 time in the control setup, the trend is only weak and not significant ( $r^2 = 0.34$ ,  $p =$   
14  $0.122$ ) due to the large spread in concentration between the two replicate samples at  
15  $t=152$  days (Fig. 4). Overall, the CL concentrations are within the same range  
16 between different experimental setups and remain essentially constant through time.  
17 Since the soils were incubated under natural light conditions, this suggests that soil-  
18 derived brGDGTs might not be sensitive to photodegradation during the incubation.

19 The contribution of IPLs to the total amount of brGDGTs in the soil at  $t=0$  is  
20  $12.4 \pm 0.3\%$  (Fig. 5), which is in the same range as has previously been reported for  
21 temperate soils from the Netherlands, Scotland, and the UK (Peterse et al., 2010;  
22 Weijers et al., 2011). The contribution of IPL-derived brGDGTs to the total pool  
23 varies mostly within the same range in the river water (7-16%), ocean water (9-15%),  
24 and control (9-17%) setups, and like the total amount of CL-brGDGTs, IPL-brGDGT  
25 concentrations show no strong trends or changes over time (river water:  $r^2 = 0.34$ ,  $p =$   
26  $0.014$ ; ocean water:  $r^2 = 0.00$ ,  $p = 0.944$ ; distilled water:  $r^2 = 0.20$ ,  $p = 0.268$ ). This  
27 suggests that brGDGTs are either produced at the same rate that they are degraded, or  
28 that production and/or degradation of brGDGTs occurs at such low rates that these  
29 processes are not detected within the timeframe of this experiment. The apparent  
30 stability of the IPL-derived, or 'living' brGDGT signature (Fig. 4) during the  
31 experiment is surprising, given the perceived lability of IPLs in general and their

1 susceptibility to loss of their headgroups within a few days after cell lysis (Harvey et  
2 al., 1986; White et al., 1979). However, previous studies have indicated that the type  
3 of headgroup and the bond through which it is attached to a core lipid may influence  
4 the rate of degradation, offering a potential explanation for the absence of changes in  
5 IPL-brGDGT concentration in our experiment. For example, after a 96 hr aerobic  
6 incubation of a mixture of IPLs with different headgroups in beach sediment, 70% of  
7 the phospholipids had degraded, compared to only 3% of the glycolipids (Harvey et  
8 al., 1986). Although a primarily glycosidic headgroup composition would be expected  
9 based on these former results, the majority of IPL-brGDGTs in soils, peat, and lake  
10 sediments are thus far identified as phospholipids (Weijers et al., 2011; Peterse et al.,  
11 2011; Tierney et al., 2012), implying that they should be sensitive to degradation,  
12 counter to our observations. **That this may not be the case may potentially be**  
13 **explained by the findings of Logemann et al. (2011), who** recently showed that the  
14 bond type within the CL, rather than the type of headgroup and its connection to that  
15 CL, determines IPL sensitivity to degradation. In their experiment, membrane lipids  
16 in which the side chains were ester-bound, as is common for bacteria, started to  
17 rapidly degrade within the first 5 days of the incubation period, whereas the  
18 abundance of lipids with ether-bound chains remained invariant during the entire 97-  
19 day experiment. BrGDGTs are thought to be produced by bacteria, but they also  
20 possess archaeal traits, including their tetraether structure (Weijers et al., 2006a). As a  
21 consequence, the presence of at least four ether bonds in each brGDGT may thus not  
22 only protect the IPL, but also the CL-brGDGTs from degradation during our  
23 experiment, even when the IPLs possess a phospho head group.

24 Weijers et al. (2010) determined a turnover rate for the total pool of CL brGDGTs in a  
25 soil of about two decades, which implies that any changes in brGDGT concentration  
26 or distribution may indeed not yet be detectable after 152 days of incubation. On the  
27 other hand, a recent study by Huguet et al. (2013) indicated that the brGDGT  
28 signature in a French peat bog had completely adapted to the 2°C maximal daytime  
29 temperature increase induced by the placement of open top mini-greenhouses within a  
30 period of less than 26 months. Although these results suggest that brGDGTs are  
31 turned over at a substantially faster rate, significant changes were only observed in the  
32 latter phase of the warming experiment, **and were primarily distributional, as their**  
33 **concentration remained the same** (Huguet et al., 2013). **The unaltered IPL-brGDGT**

1 concentrations in our experiment may thus be an indication that the time frame of our  
2 incubation has been too short to reveal any degradation processes based on IPL-  
3 brGDGT abundance. However, since the type of headgroup attached to the GDGTs  
4 has not been identified, our data does not allow to fully exclude equal production and  
5 degradation rates as possible explanation for the apparently stable IPL-brGDGT  
6 concentrations during the experiment.

7 While nutrient conditions in the river and ocean water added to the soil are  
8 presumably different, this does not seem to have had any effect on brGDGT  
9 concentrations. Moreover, given the soil:water ratio (10 g in 100 ml) used in this  
10 experiment, the amounts of nutrients that are released from the soil after the addition  
11 of water is likely substantially higher than the concentrations initially present in the  
12 river and ocean water. Nevertheless, the effect of nutrient availability on brGDGTs in  
13 soils has not yet received much attention, although recently was shown that variations  
14 in the amount and distribution of brGDGTs in lake sediments did not relate to water  
15 column nutrient concentrations (Loomis et al., 2014).

### 16 3.3 IsoGDGT concentrations over time

17 Crenarchaeol is the most abundant isoGDGT in the New Zealand soil at the start of  
18 the incubation, although its total amount (CLs + IPLs;  $63 \pm 7$  ng/g), as well as that of  
19 all other isoGDGTs ( $50 \pm 5$  ng/g) is low compared to the total pool of brGDGTs  
20 ( $239 \pm 13$  ng/g). In contrast, the average fraction of isoGDGTs initially present in the  
21 soil as IPL is 54% for crenarchaeol to 76% for isoGDGT-2, which is substantially  
22 higher than proportion of brGDGTs occurring as IPLs (12%) at the commencement of  
23 the experiment. Similar proportions of IPL-derived crenarchaeol have been found in  
24 soils from the Amazon (~50%; Zell et al., 2013b). The total concentration (IPLs +  
25 CLs) of all isoGDGTs and increases substantially during the experiment (Fig. 4), and  
26 that of isoGDGT-0 in the ocean water setup has even doubled by the end of the  
27 incubation ( $24 \pm 2$  to  $48 \pm 5$  ng/g,  $r^2 = 0.58$ ,  $p = 0.000$ ).

28 The concentrations of the individual IPL-derived isoGDGTs vary between the  
29 different incubation setups, but are overall highest in ocean water (Fig. 4;  
30 Supplementary Table). The difference in IPL-isoGDGT concentration between fresh  
31 and saline water incubations may potentially be a result of the addition of varying  
32 amounts of aquatic isoGDGTs to the soil-derived isoGDGT pool. However, 100 ml of



1 ocean water only contained ~0.5 ng isoGDGTs, which is less than 1% of their initial  
2 concentration in the soil. Alternatively, the isoGDGTs could be increasingly released  
3 from the soil matrix upon mixing with (saline) water, although the absence of a  
4 simultaneous increase in brGDGTs suggests that this is not the most likely scenario.  
5 Finally, since *Thaumarchaeota* play an important role in the nitrogen cycle in soils  
6 and the marine environment (Leininger et al., 2006; Wuchter et al., 2006; Zhang et al.,  
7 2010), nutrient availability may provide another explanation for the concentration  
8 differences between experiments. So far, crenarchaeol has been found in all  
9 (enrichment) cultures of ammonia-oxidizing *Thaumarchaeota*, and is thus considered  
10 as a biomarker lipid for this group (de la Torre et al., 2008; Pitcher et al., 2010, 2011;  
11 Sinninghe Damsté et al., 2012), although a recent study suggested that Marine Group  
12 II Euryarchaeota may also produce crenarchaeol (Lincoln et al., 2014). Nevertheless,  
13 the relatively high crenarchaeol concentration in the incubation setup using ocean  
14 water could then be a result of ammonium release due to the higher abundance of  
15 exchangeable cations (e.g. Na<sup>+</sup>, Mg<sup>+</sup>) in ocean water compared to fresh water. This is  
16 in agreement with the common method to determine available NH<sub>4</sub><sup>+</sup> in soils, which  
17 involves shaking soil in a KCl or Na<sub>2</sub>SO<sub>4</sub> solution to extract ammonium from the soil  
18 matrix (e.g. Mehlich, 1953). The concentration profiles of the other isoGDGTs are  
19 comparable to that of crenarchaeol (Fig. 4), suggesting that they are most likely also  
20 primarily derived from soil *Thaumarchaeota*.

21 Despite the significant increase of the total pools of crenarchaeol and isoGDGTs 0-3  
22 in the river ( $r^2 = 0.74, p = 0.000$  for crenarchaeol;  $r^2 = 0.56, p = 0.000$  for isoGDGTs),  
23 ocean water ( $r^2 = 0.30, p = 0.014$  for crenarchaeol;  $r^2 = 0.40, p = 0.003$  for  
24 isoGDGTs), as well as control setups ( $r^2 = 0.52, p = 0.034$  for crenarchaeol; the  
25 increase of isoGDGTs is not significant  $r^2 = 0.24, p = 0.215$ ), there is no statistically  
26 significant increase in the concentration of individual IPL-derived isoGDGTs during  
27 the course of the incubation experiment (e.g. max.  $r^2 = 0.18$  for IPL-derived  
28 crenarchaeol in ocean water; Fig. 4). We assume therefore that the production rate of  
29 IPL-isoGDGTs is approximately constant during this time. The proportional decrease  
30 of IPL-isoGDGTs with time (Fig. 5) subsequently indicates that the increase in the  
31 overall isoGDGT pool is a result of CL accumulation. This in turn confirms the  
32 general finding that the turnover of the IPL pool, and thus the release of CL-  
33 isoGDGTs, is faster than the degradation of these CLs. Regardless, the increase in



1 isoGDGT concentrations in all incubation setups suggests that availability of water is  
2 likely more important than the type of water that is added to the soil.

### 3 3.4 GDGT distribution changes

4 To evaluate if and how soil GDGT signatures are modified during the incubations, we  
5 calculated brGDGT and isoGDGT-based indices commonly used in paleoclimate and  
6 carbon cycle studies (i.e. the MBT', CBT, BIT, TEX<sub>86</sub>; Supplementary Table).

7 Next to the absence of changes in brGDGT concentration, also the MBT' and CBT  
8 indices for the ocean and river water experiments exhibit minimal variation over time,  
9 remaining within a range of 0.04 for the MBT' index, and 0.02 for the CBT (Fig. 6).  
10 Surprisingly, the largest changes are observed in the control experiment using distilled  
11 water. The MBT' index for the IPL-derived brGDGT fraction in the control setup  
12 increases from 0.23±0.00 to 0.29±0.04 ( $r^2 = 0.57$ ,  $p = 0.023$ ; Fig. 6), and is primarily  
13 caused by an increase in the concentration of brGDGT-Ia (from 23±0.3 to 29±3.9%).  
14 A closer look at the relative distributions of each of the brGDGT types at t=0 and  
15 t=152 days reveals a subtle shift in the contribution of IPL-derived brGDGT-III  
16 (decreasing from 23±1.3 to 19±0.1%) to brGDGT-II (increasing from 54±0.4 to  
17 58±0.4%) in river water (data not shown). Since both brGDGTs-II and III in the  
18 denominator of the MBT' index (Eq. 2), and the fraction of IPL-derived brGDGT-I  
19 remains stable, this change is not reflected by the index values. Given that the  
20 absolute amounts of IPL-derived brGDGTs do not significantly change with time, it is  
21 hard to determine the exact processes that cause the distributional changes, as both *in*  
22 *situ* production and preferential degradation of specific brGDGTs could influence the  
23 total concentration. Since brGDGTs with cyclopentane moieties were only present  
24 below detection limit in most of the IPL-derived fractions, it was not possible to  
25 reliably calculate CBT index values for any of these fractions.

26 The BIT index is clearly influenced by the substantial increase in crenarchaeol during  
27 the experiment, and starts to decrease between t=30 and t=91 days in all water types  
28 (Fig. 6). Due to the large percentage of crenarchaeol that is present as IPL compared  
29 to that of the brGDGTs, the BIT index of the IPL-derived fraction is much (0.48)  
30 lower than that of the CL fraction (0.88) at t=0. The lowest BIT index value for the  
31 IPL-derived fraction is 0.35 at t=152 days in ocean water (Fig. 6), and stems from the  
32 increase in crenarchaeol (and isoGDGTs in general) in this experiment. The trend of a

1 lower BIT value for the IPL versus the CL fraction has also been found for river SPM  
2 from the Yenisei (De Jonge et al., 2014) and Amazon (Zell et al., 2013b), where the  
3 difference was explained by a lower degradation rate of soil-derived crenarchaeol  
4 IPLs compared to brGDGTs and/or a contribution of *in situ* produced crenarchaeol  
5 IPLs in the river (Zell et al., 2013b). Our incubation results suggest that the  
6 degradation of IPLs from soil-derived crenarchaeol is faster than that of brGDGTs,  
7 and that the difference in BIT index between the IPL-derived and CL fractions is thus  
8 mainly caused by the higher production rate of crenarchaeol. This is in agreement  
9 with the recent finding that the BIT index of river SPM primarily tracks the seasonal  
10 aquatic production of crenarchaeol rather than that of soil input (Yang et al., 2013;  
11 Zell et al., 2013a). Our results furthermore indicate that care should be taken with  
12 using the BIT index to constrain the input of fluvially transported soil OC into a  
13 marine system. However, the stability of the soil brGDGT pool supports earlier  
14 observations that tracing the absolute amount (as opposed to the relative abundance)  
15 of brGDGTs in rivers during land-sea transport may provide a more reliable tracer of  
16 soil OC at our study site (c.f. Fietz et al., 2011; Smith et al., 2012; Zell et al., 2013b).  
17 Moreover, the spread in GDGT response factors between laboratories resulting in a  
18 range of BIT values for the same sample provides another, independent argument to  
19 use absolute concentration measurements (Schouten et al., 2013a).

20 TEX<sub>86</sub> index values start to change between 30 and 91 days of incubation for the IPL-  
21 fraction in river water and the CL-fraction in ocean water experiments (Fig. 6). We  
22 find no clear explanation for the diverging trends for the different water types or IPL  
23 versus CL fractions, but there are several factors that may contribute to the observed  
24 changes. For example, shifts in the initial isoGDGT-community due to water contact,  
25 different membrane adaptation rates, or preferential release from the soil matrix may  
26 all have occurred. Alternatively, the relative distribution of isoGDGTs may differ per  
27 type of headgroup in both marine (Schouten et al., 2008; Pitcher et al., 2011; Lengger  
28 et al., 2012) and soil *Thaumarchaeota* (Sinninghe Damsté et al., 2012), so that the  
29 deviation in TEX<sub>86</sub> values may also be explained by different turnover rates per  
30 headgroup. However, even though the total variation in TEX<sub>86</sub> corresponds with a  
31 4°C SST change in the experiment, given the low absolute amounts of terrestrial  
32 isoGDGTs that are finally discharged to the ocean in this case, this will have a minor

1 impact on TEX<sub>86</sub> records from the marine environment where their abundances are  
2 much higher.

### 3 **3.5 Experimental setup evaluation**

4 Despite prior evidence for in situ production of brGDGTs in several large rivers (e.g.  
5 Kim et al., 2012; Zhang et al., 2012; Zell et al., 2013b; Yang et al., 2013; De Jonge et  
6 al., 2014), we have not observed this in our experiment. The absence of an in situ  
7 production signal in our data may be a consequence of the soil:water ratio of 1:10 that  
8 we used in our experimental setup, which is likely more concentrated than in most  
9 natural systems, so that the relatively large amount of soil-brGDGTs may have  
10 overprinted any evidence of aquatic brGDGT production during the experiment. An  
11 indication that in situ production in the Rakaia River may take place after all is  
12 reflected by the offset between brGDGTs in river SPM and that in the soil (Fig. 6).  
13 Although this soil-SPM offset is based on a single sample location in the entire river  
14 basin and brGDGT distributions in soils may show substantial variation within a small  
15 area (e.g. Weijers et al., 2007b; Naeher et al., 2014), it may also support the idea that  
16 brGDGTs in soils and river SPM may have different sources. Notably, also the  
17 brGDGT distributions in river and ocean SPM show an offset (Fig. 6), despite the  
18 ocean water sample location close to the river mouth. Differences in biomarker  
19 distributions in SPM from the fresh-saline water transition have previously been  
20 attributed to hydrological sorting (e.g. Goñi et al., 1998), or, more specifically in case  
21 of brGDGTs, additional marine production (e.g. Zhu et al., 2011; Zell et al., 2014). In  
22 our case, this offset may have also been introduced by the use of filters with different  
23 pore size to obtain the SPM from the river and ocean water (0.2 µm and 0.7 µm,  
24 respectively). This has resulted in a comparison of ‘free living’ and ‘suspended’ lipid  
25 fractions, which at least in marine water may have a different composition (Ingalls et  
26 al., 2012; Close et al., 2014).

27 Furthermore, 152 days of incubation appears not to have been long enough to  
28 determine the sensitivity of brGDGTs to degradation in aquatic environments based  
29 on changes in their distribution or abundance. The unexpected apparent stability of  
30 the IPL-derived brGDGTs suggests that the presence of IPL-brGDGTs in aquatic  
31 systems does not necessarily indicate that they are produced in situ, and rather support  
32 the earlier finding that IPLs containing ether lipids are relatively resistant against

1 degradation, even on a longer time scale than has previously been shown (t=97 days;  
2 Logemann et al., 2011). Alternatively, the water-saturated conditions may have  
3 inhibited both growth and degradation of brGDGTs to take place. However, without  
4 analyzing the exact headgroup composition of the brGDGTs in our experiment it is  
5 not possible to distinguish between these processes.

6 For this study, it was assumed that the soil-derived (IPL-)GDGTs would be degraded  
7 in aquatic environments, yet our data indicate that the concentration of isoGDGTs  
8 actually increased over time in all setups (Fig. 4). This suggests that isoGDGTs are  
9 either produced, or that they are (preferentially) released from the soil matrix during  
10 the experiment. In order to get a better control on the exact processes taking place, the  
11 control setup could have benefitted from the addition of a chemical agent to prevent  
12 any microbial activity, and thus to avoid additional GDGT-production during the  
13 experiment.

14 To summarize, follow-up experiments could benefit from a more natural soil:water  
15 ratio, so that a potential contribution of aquatic GDGTs can be detected by analyzing  
16 the soils. The control setup should be treated to prevent any microbial activity. In  
17 addition, determining the abundance and distribution of GDGTs present in the  
18 incubation water could likely provide further information on water column processes,  
19 provided that the amount of water added to the soil is sufficient to obtain a large  
20 enough sample upon filtering for GDGT analysis. Finally, to better understand the  
21 apparent stability of IPL-brGDGTs in aquatic environment, the type of headgroup  
22 should be monitored during the incubation experiment, which should run longer than  
23 the 152 days of this study.

#### 24 25 **4 Conclusions**

26 Laboratory incubation experiments involving admixture of soil and fresh/ocean water  
27 indicate that soil-derived brGDGTs appear to be surprisingly stable in aquatic  
28 environments in both IPL- and CL configurations. Our observations suggest that soil  
29 brGDGT signatures will likely be unaltered during fluvial transport from land to sea.  
30 However, distributional offsets between brGDGTs in river SPM and catchment soil,  
31 as well as between brGDGTs in river SPM and ocean SPM indicate that additional  
32 sources may contribute to the final distribution in which brGDGTs are delivered to

1 **the ocean.** This means that the exact sources of brGDGTs in a river system need to be  
2 well constrained before these compounds can be used as reliable tracers for land-sea  
3 transport of soil organic carbon, as well as for paleoclimate reconstructions.

4 As opposed to brGDGTs, the concentration of isoGDGTs increased during the  
5 incubation experiments, altering their initial relative distribution. Although the  
6 changes in TEX<sub>86</sub> index values correspond with a maximum change of about 4°C in  
7 reconstructed SSTs in the ocean water setup, the low abundance of isoGDGTs in soils  
8 relative to in marine settings suggests that their land-sea transport would have  
9 minimal impact on TEX<sub>86</sub> recorded in the marine sedimentary record. Nevertheless,  
10 after 30 days of incubation, the increase in isoGDGTs was sufficient to affect BIT  
11 index values. This may have consequences for the interpretation of TEX<sub>86</sub>-based SST  
12 records with a BIT index close to the cutoff value of 0.3 (Weijers et al., 2006b), as the  
13 actual input of soil-derived GDGTs upon delivery to the ocean may be larger than  
14 anticipated.

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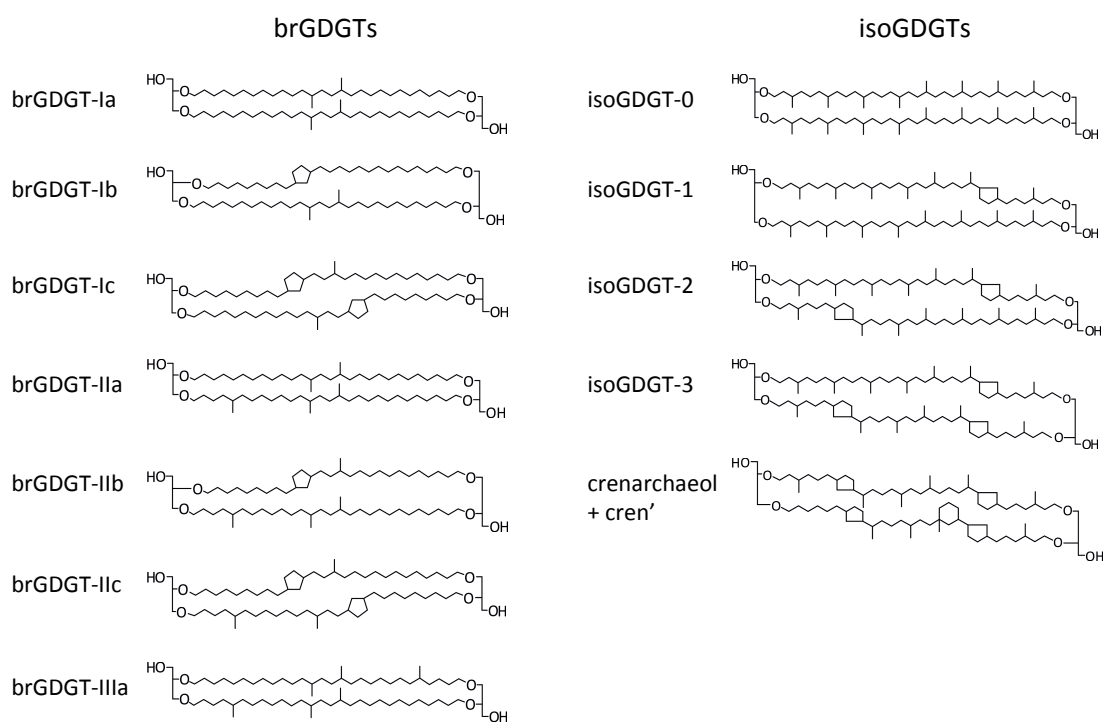
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2 Figure 1. Molecular structures of the brGDGTs and isoGDGTs monitored during the  
3 incubation experiment.

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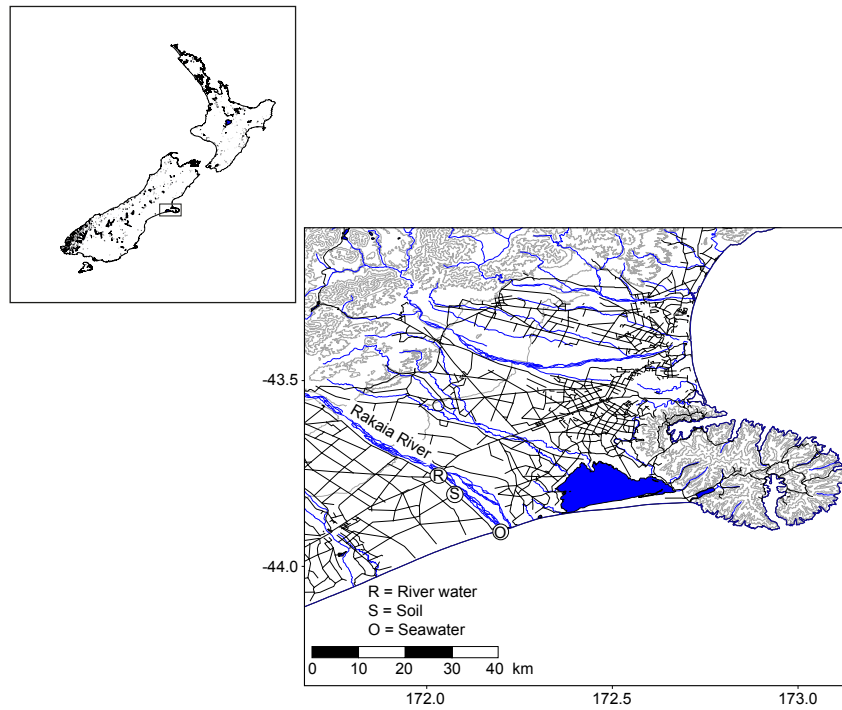
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1 Figure 2. Overview map of the Rakaia River, South Island, New Zealand, indicating  
2 the sampling locations.

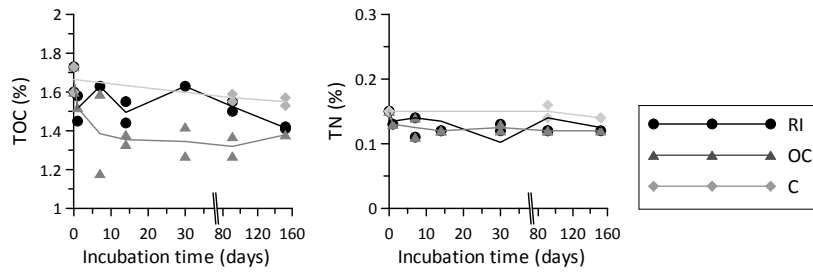
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1 Figure 3. Total organic carbon (TOC) and total nitrogen (TN) concentrations in a  
2 sandy loam soil from the Raiaka River catchment, New Zealand, during incubation (t  
3 = 152 days) in river (RI), ocean (OC), and distilled (C) water.  
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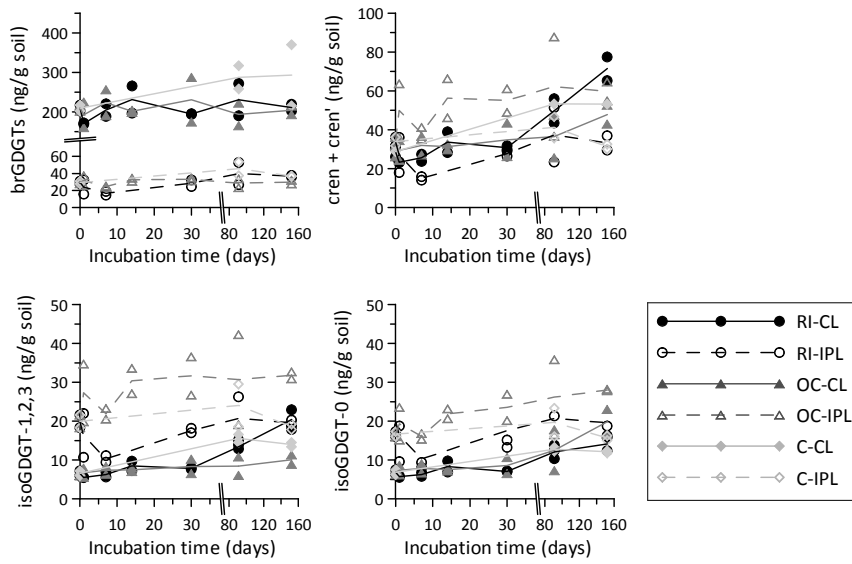


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2 Figure 4. Concentrations of core lipid (CL) and intact polar lipid (IPL)-derived a)  
3 brGDGTs, b) crenarchaeol + cren', c) isoGDGT-1,2,3, and d) isoGDGT-0 in a sandy  
4 loam soil from the Raiaka River catchment, New Zealand, during incubation (t = 152  
5 days) in river (RI), ocean (OC), and distilled (C) water. Average concentrations are  
6 indicated by solid (CLs) and dashed (IPL-derived) lines.

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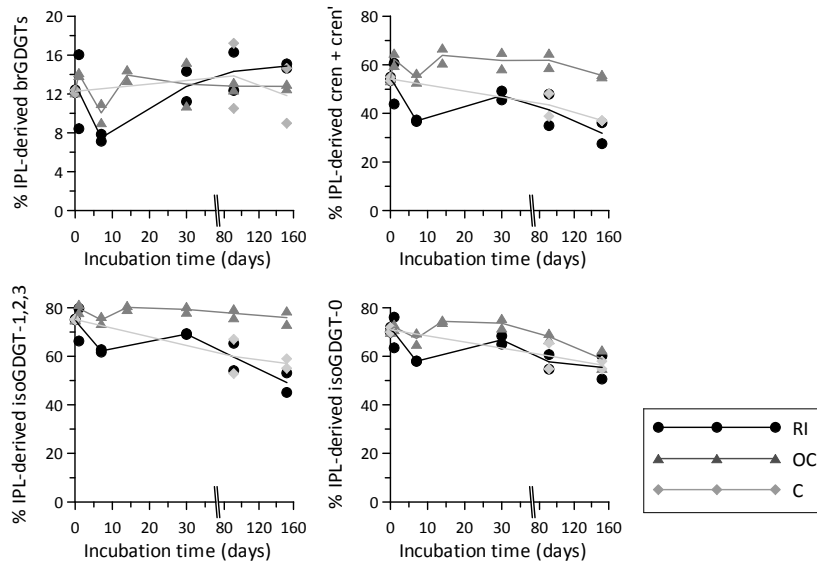


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2 Figure 5. Percentages of the total pool of a) brGDGTs, b) crenarchaeol + cren', c)  
3 isoGDGTs-1,2,3, and d) isoGDGT-0 present in 'living', or intact polar lipid (IPL)-  
4 derived form. Average concentrations are indicated by solid lines.

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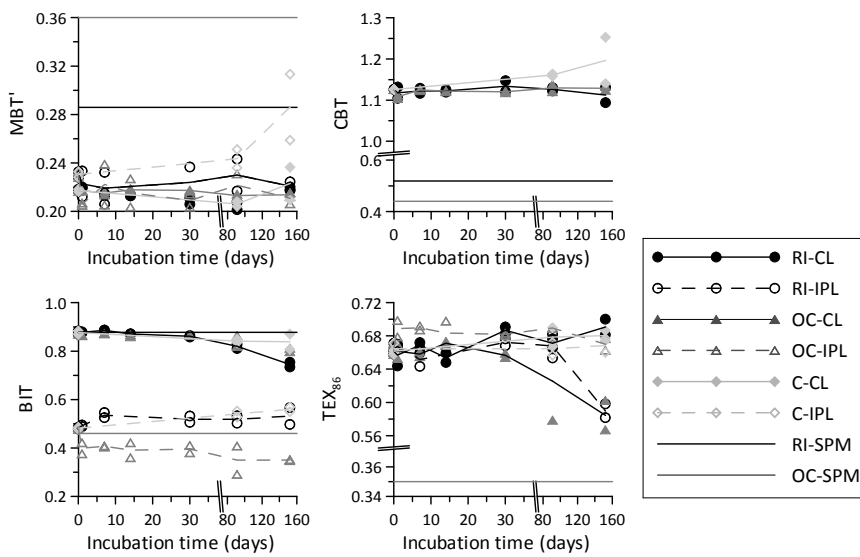
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2 Figure 6. Distribution of core lipid (CL) and intact polar lipid (IPL)-derived GDGTs  
3 in a sandy loam soil from the Rakaia River catchment, New Zealand, as reflected by  
4 the a) MBT' index, b) CBT index, c) BIT index, and d) TEX<sub>86</sub> index during  
5 incubation (t = 152 days) in river (RI), ocean (OC), and distilled (C) water under  
6 quasi-aerobic conditions. Horizontal straight lines represent GDGT composition in  
7 river (black) and ocean water (grey) suspended particulate material (SPM). Average  
8 concentrations are indicated by solid (CLs) and dashed (IPL-derived) lines.  
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