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

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

1 Introduction

The global carbon cycle encompasses a myriad of biogeochemical processes that influence our climate and link all carbon reservoirs on the Earth surface. Soils are considered to play a very active and fundamental role in this cycle, as their mobilization from land and subsequent deposition in marine sediments serves as a long-term sink of atmospheric CO_2. However, the exact magnitude and mechanisms of soil organic carbon (OC) transfer from terrestrial source to marine sink remain elusive (e.g. Cole et al., 2007; Weyhenmeyer et al., 2012), which partly stems from the lack of a suitable tracer of the soil OC pool. Our current insights in land-sea soil OC transport dynamics are primarily based on bulk properties of river suspended particulate matter (SPM). However, next to soil OC, river SPM also comprises carbon derived from aquatic production and 'fossil' carbon from rock erosion, each of which influences the bulk properties of SPM (Blair et al., 2010).

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

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

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

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

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

2 Materials and methods

2.1 Sample collection and incubation setup

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

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

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

2.2 Total organic carbon and nitrogen analysis

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

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

2.3 GDGT extraction and analysis

Freeze-dried soil samples (3-4 g) were solvent-extracted three times using a Bligh and Dyer technique modified from Sturt et al. (2004). In short, a solvent mixture of MeOH:dichloromethane (DCM): phosphate buffer at pH 7.4 (2:1:0.8, v/v/v) was added to the soils and ultrasonically extracted for 10 min. The extract was collected each time after centrifuging at 1000 rpm for 5 mins. DCM and phosphate buffer were added to the combined extracts to a volume ratio of 1:1:0.9 to obtain phase separation. The DCM phase, containing the GDGTs, was collected after centrifuging, and the remaining solvent was rinsed twice with DCM. The combined DCM phases were dried under N_2 and passed over a silica column (deactivated with 1 weight% water) to separate CLs and IPLs according to (Pitcher et al., 2009), with the exception that hexane:ethyl acetate 1:1 (v/v) was used to elute the CLs. An aliquot of the IPL fraction was analyzed directly for CLs in order to assess potential carry over. The remainder of IPL fraction was dissolved in 6N HCl in MeOH and heated at 100°C for at least 3h to release IPL-bound CLs. A known amount of C_{46} GDGT standard (Huguet et al., 2006) was added to all fractions prior to analysis.

The filters were freeze dried and extracted with a MARS Xpress microwave extraction system, using DCM:MeOH 9:1 (v/v). After centrifugation (5 min at 400 rpm), the total lipid extract (TLE) was pipetted off and the residues were rinsed twice with DCM:MeOH 9:1 (v/v). The combined extracts were dried under N_2 with a known amount of C_{46} GDGT standard, after which the TLEs were separated into an apolar and a polar (GDGT) fraction by passing them over a silica (1% water deactivated) column using hexane:DCM 9:1 (v/v) and DCM:MeOH 1:1 (v/v), respectively.

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

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

2.4 GDGT-based index calculations

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

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

and (Peterse et al., 2012):

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

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

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

and for the TEX_{86} index based on isoGDGTs the equation of (Schouten et al., 2002) was used:

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

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

3 Results and discussion

3.1 TOC and TN concentrations over time

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

3.2 BrGDGT concentrations over time

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

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

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

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

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

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

3.3 IsoGDGT concentrations over time

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

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

ocean water only contained ~0.5 ng isoGDGTs, which is less than 1% of their initial concentration in the soil. Alternatively, the isoGDGTs could be increasingly released from the soil matrix upon mixing with (saline) water, although the absence of a simultaneous increase in brGDGTs suggests that this is not the most likely scenario. Finally, since *Thaumarchaeota* play an important role in the nitrogen cycle in soils and the marine environment (Leininger et al., 2006; Wuchter et al., 2006; Zhang et al., 2010), nutrient availability may provide another explanation for the concentration differences between experiments. So far, crenarchaeol has been found in all (enrichment) cultures of ammonia-oxidizing *Thaumarchaeota*, and is thus considered as a biomarker lipid for this group (de la Torre et al., 2008; Pitcher et al., 2010, 2011; Sinninghe Damsté et al., 2012), although a recent study suggested that Marine Group II Euryarchaeota may also produce crenarchaeol (Lincoln et al., 2014). Nevertheless, the relatively high crenarchaeol concentration in the incubation setup using ocean water could then be a result of ammonium release due to the higher abundance of exchangeable cations (e.g. Na^+, Mg^+) in ocean water compared to fresh water. This is in agreement with the common method to determine available NH_4^+ in soils, which involves shaking soil in a KCl or Na_2SO_4 solution to extract ammonium from the soil matrix (e.g. Mehlich, 1953). The concentration profiles of the other isoGDGTs are comparable to that of crenarchaeol (Fig. 4), suggesting that they are most likely also primarily derived from soil *Thaumarchaeota*.

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

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

3.4 GDGT distribution changes

To evaluate if and how soil GDGT signatures are modified during the incubations, we calculated brGDGT and isoGDGT-based indices commonly used in paleoclimate and carbon cycle studies (i.e. the MBT', CBT, BIT, TEX_{86}: Supplementary Table).

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

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

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

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

impact on TEX_{86} records from the marine environment where their abundances are much higher.

3.5 Experimental setup evaluation

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

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

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

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

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

4 Conclusions

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

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

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

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

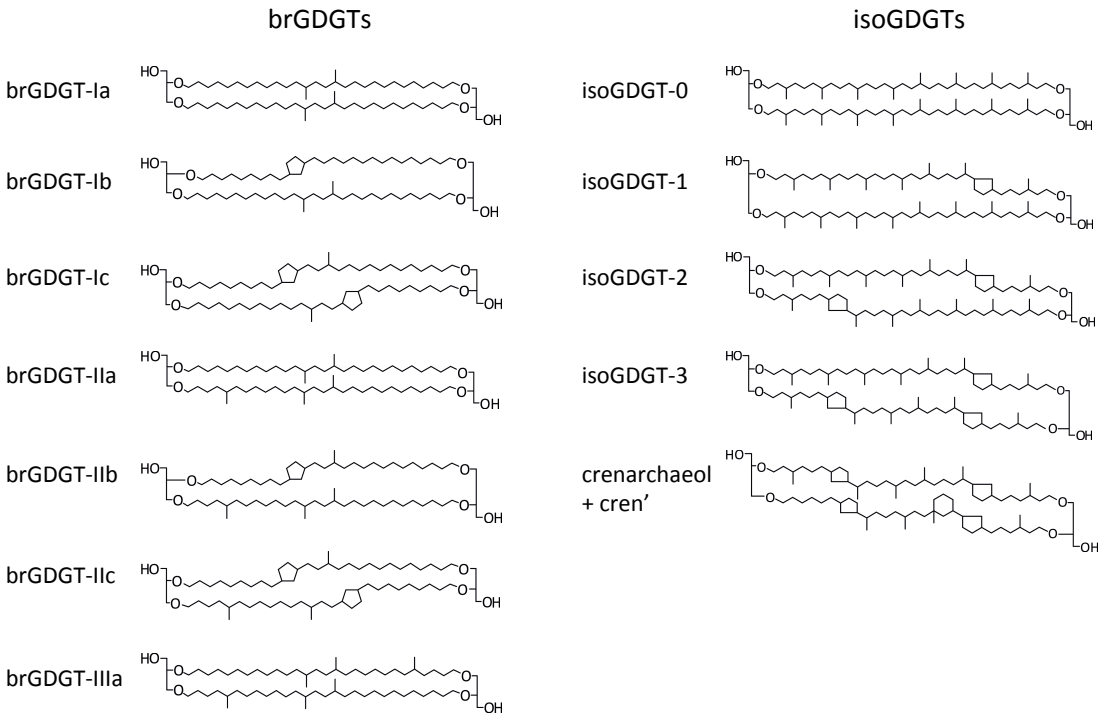

Figure 2. Overview map of the Rakaia River, South Island, New Zealand, indicating the sampling locations.

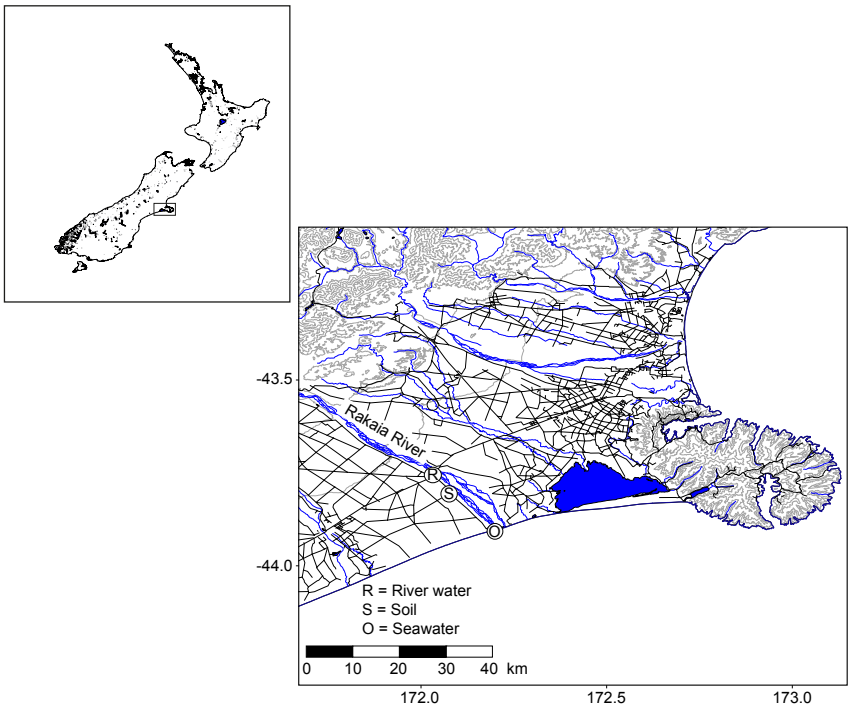

Figure 3. Total organic carbon (TOC) and total nitrogen (TN) concentrations in a sandy loam soil from the Raiaka River catchment, New Zealand, during incubation (t = 152 days) in river (RI), ocean (OC), and distilled (C) water.

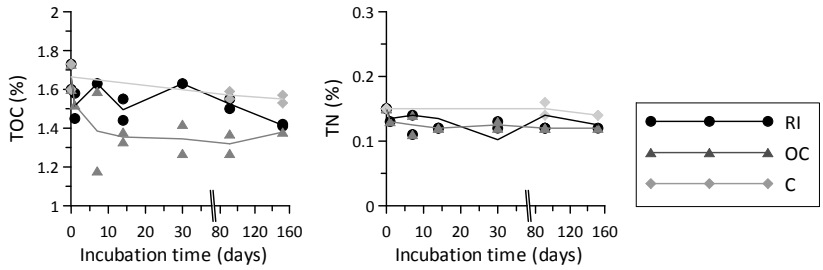

Figure 4. Concentrations of core lipid (CL) and intact polar lipid (IPL)-derived a) brGDGTs, b) crenarchaeol + cren', c) isoGDGT-1,2,3, and d) isoGDGT-0 in a sandy loam soil from the Raiaka River catchment, New Zealand, during incubation (t = 152 days) in river (RI), ocean (OC), and distilled (C) water.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

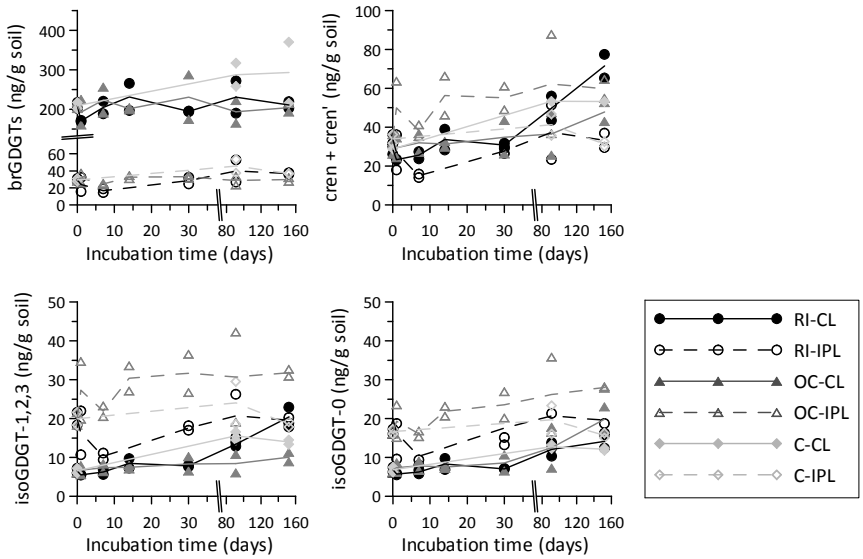

Figure 5. Percentages of the total pool of a) brGDGTs, b) crenarchaeol + cren', c) isoGDGTs-1,2,3, and d) isoGDGT-0 present in 'living', or intact polar lipid (IPL)-derived form. Average concentrations are indicated by solid lines.

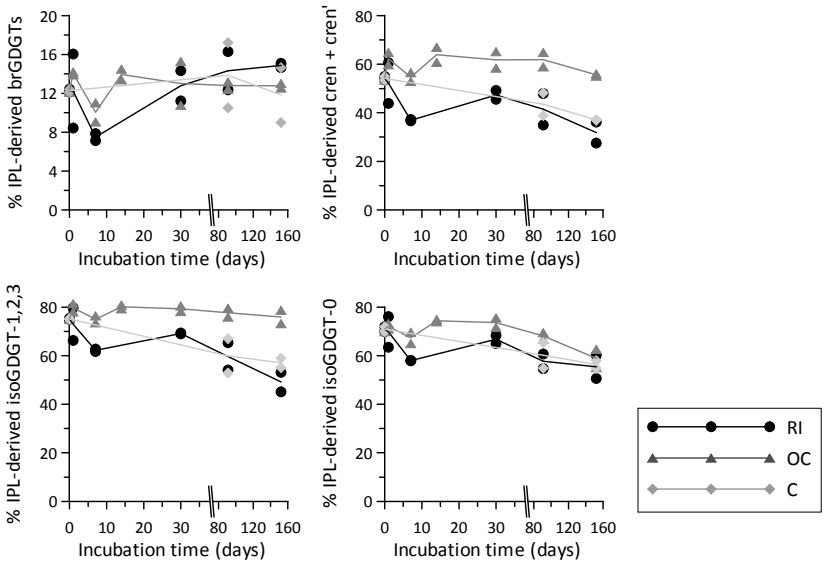

Figure 6. Distribution of core lipid (CL) and intact polar lipid (IPL)-derived GDGTs in a sandy loam soil from the Rakaia River catchment, New Zealand, as reflected by the a) MBT' index, b) CBT index, c) BIT index, and d) TEX_{86} index during incubation (t = 152 days) in river (RI), ocean (OC), and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