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Abstract

Most organic matter (OM) on Earth occurs as kerogen-like materials, i.e. naturally formed macromolecules insoluble with standard organic solvents. The formation of this insoluble organic matter (IOM) is a topic of much interest, especially when it limits the detection of compounds of geomicrobiological interest. For example, studies that search for biomarker evidence of life on early Earth or other planets usually use solvent-based extractions. This leaves behind a pool of OM as unexplored post-extraction residues, potentially containing diagnostic biomarkers. Since the IOM has an enhanced potential for preservation compared to soluble OM, analysing IOM-released biormarkers can also provide even deeper insights into the ecology of ancient settings, with implications for early Earth and Astrobiology investigations. Here, we analyse the prokaryotic lipid biosignature within soluble and IOM of the Taupo Volcanic Zone (TVZ) silica sinters, which are key analogues in the search for life. We apply sequential solvent-extractions and a selective chemical degradation upon the post solvent-extraction residue. Moreover, we compare the IOM from TVZ sinters to analogous studies on peat and marine sediments to assess patterns in OM insolubilisation across the geosphere. Consistent with previous work, we find significant but variable proportions – 1–45% of the total prokaryotic lipids recovered – associated with IOM fractions. This occurs even in recently formed silica sinters, likely indicating inherent cell insolubility. Moreover, archaeal lipids seem more prone to insolubilisation as compared to the bacterial analogues, which might enhance their preservation and also bias overall biomarkers interpretation. These observations are similar to those observed in other settings, confirming that even in a setting where the OM derives predominantly from prokaryotic sources, patterns of IOM formation/occurrence are conserved. Differences with other settings, however, such as the occurrence of archaeol in IOM fractions, could be indicative of different mechanisms for IOM formation that merit further exploration.

Key words: Taupo Volcanic Zone, silica sinters, early Earth, Astrobiology, selective chemical degradation, prokaryotic membrane lipids

1. Introduction

Most of the OM on Earth occurs in the geosphere as kerogen-like materials in the form of IOM (Berger, 1989; Harvey, 2006), i.e. OM that is non soluble with standard solvent-based extractions (e.g. Rullkötter and Michaelis, 1990; Vandenbroucke and Largeau, 2007). OM has been reported to persist in the geosphere for up to 3,770 million years (Dodd et al., 2017), with lipids having the highest preservation potential of all biomolecules (Tissot and Welte, 1984; Tegelaar et al., 1989) and therefore, representing one of the most direct biosignatures of life on the early Earth (Summons et al., 2008) and potentially in the solar system (Schuerger and Clark, 2008). Such are the insights about life

on early and beyond Earth that can be gained from lipids that the NASA 2015 Astrobiology Strategy includes them as part of the biosignatures that can be identified, explored and characterized in the search for habitability in extraterrestial environments. Consequently, lipid biomarkers are extensively used to explore the Earth´s past and current ecology (e.g. Ward et al., 1989; Summons et al., 1996; Freeman, 2001; Hayes, 2001; Simoneit, 2002; Orphan et al., 2008; Dreier et al., 2012; O´reilly et al., 2017) and are target molecules (alongside other organics) in the search for life fingerprints in extraterrestrial or analogous environments (e.g. Summons et al., 2011; Bühring et al., 2012; Mahaffy et al., 2012; Leshin et al., 2013; Hays et al., 2017; Tan et al., 2018).

Since it is well stablished that most of the Earth´s OM is insoluble with standard solvent-extractions (Berger, 1989; Harvey, 2006), many studies have examined the lipid biosignatures of the IOM fractions. For example, some work has explored the organic components that are covalently cross-linked via sulfur species (e.g. Sinninghe Damsté and de Leeuw, 1990; van Dongen et al., 2003; 2006) or via oxidative cross-linking (Harvey et al., 1983; Versteegh et al., 2004). Other studies release organics that have likely become insoluble via random polymerisation reactions (Tissot and Welte, 1984), such as esterification, glycosidic or amide bond formation (e.g. Naafs and van Bergen, 2002; Otto and Simpson, 2007; Allard and Derenne, 2009; Weijers et al., 2011; Chaves and Pancost, 2016). And in many other investigations the IOM is assessed by performing pyrolysis, applied to a wide range of environmental settings on Earth (e.g. Martín et al., 1995; Largeau et al., 1986; Derenne and Quénéa, 2015) and even on Mars (Ming et al., 2013) or Mars analogous settings (Lewis et al., 2018). Despite these many studies that take into account the IOM fractions, the majority of investigations that search for evidence of life as lipid biosignatures – on or beyond Earth – are typically constrained to soluble OM fractions (e.g. Zeng et al., 1992; Brocks et al., 1999; Pancost et al., 2005; Turich et al., 2007; Kaur et al., 2008; Bühring et al., 2012; Tan et al., 2018). This leaves behind a whole niche of unexplored IOM in a wide range of geological settings. Moreover, the IOM has an enhanced preservation potential – due to a diminished surface access that affords protection against chemical and microbial degradation (Durand, 1980; Tissot and Welte, 1984) – and therefore, additional insights are expected from the analysis of well-preserved lipids released from the IOM. For example, sterane biomarkers in hydropyrolysates of Neoproterozoic rocks provided first insights into the appearance of

the first animals on Earth (Love et al., 2009; Zumberge et al., 2018). Moreover, assessing the IOM can overcome misinterpretation of Precambrian organic geochemistry due to contamination risks (French et al., 2015).

Hot spring silica deposits – due to their harsh environmental conditions – are a natural habitat for hyperthermophilic prokaryotes and, therefore, key early Earth analogues (Walter, 1972; Konhauser et al., 2001; Konhauser et al., 2003; Phoenix et al., 2006). In addition, microbial communities can be mineralised via silica supersaturation of geothermal fluids, which turns the original siliceous stromatolites into biofacies that can be preserved for billions of years (Konhauser, 2003; Djokic et al., 2017). Consequently, ancient microbial communities can be analysed for morphological and molecular biosignatures of life on early Earth or beyond Earth analogues (Schultze-Lam et al., 1995; Konhauser, 2003; Djokic et al., 2017). Moreover, silica sinters forming in currently-active hot springs can also be used as analogues for Precambrian siliceous stromatolites (Konhauser et al., 2001) or even analogous extraterrestrial settings (e.g. Walter and Des Marais, 1993; Ruff and Farmer, 2016).

In the current study, therefore, we explore the lipid distributions in both the soluble and IOM of TVZ hot spring silica sinters. This work complements previous investigations on the distribution of prokaryotic membrane lipids in the TVZ, which focused strictly on the solvent-extractable OM fractions and their geochemical implications (Pancost et al., 2005; Kaur et al., 2008; 2011a; 2011b; 2015). In particular, we focus on the prokaryotic biomarkers that occur in the IOM fractions and explore which biomarkers and associated organisms might be more prone to preservation. We apply sequential solvent-extractions to obtain soluble OM and compare it to IOM obtained after selective chemical degradation of the post solvent-extraction residue – sequential base and acid hydrolysis. Most of the OM in the TVZ silica sinters – due to the high temperature and low pH of the setting – is of extremophile origin (Kaur et al., 2008; 2011a; 2011b; 2015). We target specifically (see Appendix) *iso-* and *anteiso-* C_{15} and C_{17} FAs (branched FAs; I – IV); C_{31}-C_{33} hopanoic acids (V) and anhydrobacteriohopanetetrol (anhydroBHT; VI); DAGEs (VII); archaeol (VIII); i-GDGTs (IX – XIV), which facilitates the assignment of chemical structures to the biological source since such lipids have relatively diagnostic structures (Kaneda, 1991; Kannenberg and Poralla, 1999; Koga and Morii,2007). This represents only a small percentage (<3.5%) of the total organic carbon (TOC) of the sinters (see

Supporting information), but it is crucial for understanding the partitioning of these biomarkers. Finally, the use of prokaryotic lipids as biomarkers in the current study enables a comparison to previous work on peat (Chaves Torres and Pancost, 2016) and marine sediments (Chaves Torres et al., 2017), and therefore, to assess whether previous findings apply in a setting where there is no wider OM matrix, i.e. whether previously observed patterns for IOM occurrence/formation are consistent across the geosphere.

2. Methods

2.1. TVZ silica sinters

Silica sinters were collected from different TVZ geothermal systems, Champagne Pool (CP; CP1 and CP2) and Opaheke Spring (OP) (Table 1), on the North Island of New Zealand. The deposits are recent, ranging from active sinters (<10 y; CP1and OP) to an inactive sinter (900 y; CP2); the age is estimated from the rate of silica precipitation and distance from the pool edge (Kaur et al., 2008). The chemical composition of the sinters is mainly amorphous silica, with diverse trace elements, such as Au, Ag, Sb, W (and As in the case of Champagne Pool, CP1 and CP2; Jones et al., 2001), also being present. The arsenic and antimony sulfides in CP1 and CP2 are responsible for the distinct orange colour of these samples (Phoenix et al., 2005) as opposed to the Opaheke Spring (OP) sample, which is grey. CP and OP also differ in that the latter was deposited at somewhat higher temperatures (Table 1). The OM content of all three samples is very low – TOCcontents are typically less than 1% (Table 1) – and almost exclusively derived from prokaryotic sources; for further site and sample details see Kaur et al. (2008; 2011a; 2011b; 2015).

2.2. Experimental procedure

Prior to the application of the analytic scheme, silica sinters were pre-extracted in DCM/MeOH (1:1, v/v) for 10 mins to test external contamination. This treatment ensures that subsequent work-up yields compounds likely derived from microorganisms encased in the silica matrix (Pancost et al., 2005). Silica sinters were then air-dried, ground and homogenised. TOC of the sinters was calculated as the difference between total carbon (TC) and total inorganic carbon (TIC), with TC determined with a Carlo Erba EA1108 elemental analyser and TIC using a modified Strohlein Coulomat 702 analyser.

The overall analysis of the ground samples consisted of sequential solvent-extractions and a selective chemical degradation of the post-extraction residue, as has been described in detail elsewhere (Chaves Torres and Pancost, 2016). Briefly, ground silica sinters (Table 1) were extracted via sonication in a Bligh and Dyer extraction mixture (BD) (3×) – buffered water (solution of 0.05M KH_2PO_4 in water, adjusted to pH 7.2 with NaOH), $CHCl_3$ and MeOH (4:5:10, v/v). The extract obtained from BD, E1BD, was subjected to acid methanolysis (AMe) – a reflux of 2N HCl, 86% in MeOH, for 3h at 100 °C – to remove intact polar (IP) head groups. E1BD was then kept for analysis and the air-dried residue, R1BD was further solvent-extracted with Soxhlet 48h (Sox) in DCM/MeOH (2:1, v/v). The extract obtained from Sox, E2Sox, was analogously subjected to AMe and then kept for analysis and the air-dried residue, R2Sox, was kept for chemical degradation. Prior to instrumental analysis of the solvent extracts – E1BD and E2Sox – free sulfur was removed with HCl_{aq} activated copper.

Chemical degradation upon R2Sox consisted of two sequential hydrolysis steps. First, base hydrolysis (BHy) – 1M KOH, 96% in MeOH, 1h at 70 °C – aiming to release ester-bound moieties from the IOM. The BHy extract, E3BHy, was then obtained and the post BHy residue, R3BHy, was exhaustively solvent-extracted with a sequence of decreasing polarity solvents (H_2O, H_2O/MeOH, MeOH and DCM) to ensure no base-hydrosylates remained in R3BHy. Subsequent solvent extracts were then combined with E3BHy, methylated with a reflux of BF_3, 14% in MeOH, for 30 min at 60 °C, and then kept for analysis. R3BHy was air-dried and subjected to AMe as described above, which aimed to cleave amide-, glycosidic- and remaining ester-bound moieties from the IOM. Analogously to R3BHy solvent washing, the AMe residue, R4AMe, was exhaustively solvent-extracted with H_2O, H_2O/MeOH, MeOH and DCM to remove any acid-hydrosylates within the residue. Subsequent extracts were then combined with E4AMe and it was kept for analysis. Hydroxyl groups within all extracts – soluble (E1BD and E2Sox) and insoluble (E3BHy and E4AMe) – were made amenable to GC via trimethylsilylation (TMS-derivatization) prior to GC-MS analysis. Additionally, i-GDGTs of all extracts were analysed via HPLC-MS. All instrumental details for lipid analysis are published elsewhere (Chaves Torres and Pancost, 2016). We target particular prokaryotic lipids, such as (see Appendix) branched FAs (I –IV), hopanoids (V, VI), DAGEs (VII), archaeol (VIII) and i-GDGTs (IX – XIV).

3. Results

Biomarkers identified in the soluble (BD and Sox) and IOM (BHy and AMe) fractions of the CP and OP silica sinters appear to be primarily of prokaryotic origin (Fig. 1; Supporting information Figures S1 and S2). Additionally, a minor proportion of *n*-alkanes also occur and are attributed to allochthonous higher plant input (Eglinton et al., 1962; Kaur, 2008). Intriguingly, *n*-alkanes are observed in solvent-extractable fractions, but also in insoluble fractions, consistent with previous investigations (e.g. Amblès et al., 1996). A thorough analysis of the solvent-extracted lipid biomarkers, including FAs, hopanoids, DAGEs, archaeol and i-GDGTs, that occur in these geothermal settings has been reported elsewhere (Kaur, 2008a; 2008b; Kaur et al., 2011a; 2011b; 2015). Those papers generally focused on the neutral fractions, i.e. the free lipid biomarkers rather than the IP lipids, such that a direct comparison to this work, where all solvent-extractable lipids will have been released via AMe of total lipid extracts (TLEs), is not appropriate. However, we include the yields of the biomarkers of interest from this study in the Supporting information Tables S1, S2 and S4, as well as the distribution of total compounds among OM fractions (Supporting information Figure S3). The aforementioned papers comprehensively covered interpretations associated with these lipid classes, including likely sources and variations among settings. Consequently, the following sections focus solely on concentrations and proportions of (see Appendix) branched FAs (I – IV), hopanoids (V, VI), DAGEs (VII), archaeol (VIII) and i-GDGTs (IX – XIV) that occur in the soluble OM fractions as compared to the IOM fractions (Supporting information Table S3). No br-GDGTs were detected in any of the silica sinters extracts.

It is important to note that the analytical procedures used, although more comprehensive than standard biomarker extractions, are likely still non-exhaustive. By extracting via both BD and Sox, as well as hydrolysing the resulting TLEs, we are confident that the majority of the soluble biomarkers have been identified. That is likely not true of the IOM. Although the chemical degradation techniques used here are standard and expected to be relatively exhaustive, steric hindrance, especially in complex geomacromolecular IOM, could partially inhibit reaction efficiency. Moreover, it is possible that these techniques, especially BHy, release lipids that retain their polar head groups, i.e. GDGTs with glycosyl moieties, which would not be detected via subsequent analyses. Consequently, yields of biomarkers from IOM – although clearly statistically significant in the current results – are likely to be

minimum values. It is also possible that if such biases exist, they differ among compound classes imparting different apparent patterns of OM insolubilisation.

3.1. Branched FAs

Concentrations of branched FAs (I – IV) relative to TOC are highly variable, ranging from 0.024 to 0.93 mg g^{-1} TOC at OP (Fig. 2a). Similarly, the concentration ratios of branched relative to the straight-chain C_{15} and C_{17} FAs range from 0.13 (CP2) to 15 (OP) (Fig. 2b). In all three samples, the yields of the first solvent-extraction (BD) are higher than the sequential Sox yields. However, 11–35% of the total branched FAs recovered occur in the sequential Sox extracts, and 14–45% of branched FAs occur in IOM fractions. The IOM fractions in the OP sinter are dominated by branched FAs released after AMe rather than BHy (4% BHy vs. 21% AMe extracts). This contrasts markedly with the CP sinters, in which the insoluble branched FAs occur predominantly in the BHy extracts: 33% BHy vs. 12% AMe extracts (CP1) or 14% BHy vs. none in the AMe extracts (CP2). Additionally, there are complex distributions of FAs among soluble and IOM fractions. For example, the ratio of branched vs. straight-chain FAs (Fig. 2b) is higher in IOM fractions from CP2 (1.7 times higher in BHy fractions) and OP (1.5 times higher in BHy fractions and 2.9 times higher in AMe fractions), but lower in IOM fractions of CP1 (1.7 times lower in BHy and 2.4 in AMe extracts).

3.2. Hopanoids

Series of C_{31} to C_{33} geohopanoids (V) occur in the BD extracts, predominantly occurring as 17β,21β(H) hopanoic acids, but with the 17α,21β(H)- and 17β,21α(H) analogues also present. AnhydroBHT (VI; Schaeffer, 1993; Saito and Suzuki, 2007) also occurs, consistent with previous work (Gibson et al., 2014). Overall, concentrations of hopanoids range from 0.11 (OP) to 2.3 mg g^{-1} TOC at CP1 (Fig. 3a), and no geohopanoids were detected in the active CP1, consistent with their origin from diagenetic reactions over time (Innes et al., 1997; Innes et al., 1998; Farrimond et al., 2002; Saito and Suzuki, 2007). The majority of geohopanoids occur as extractable lipids (71–92%), consistent with the behaviour of the branched FAs (Section 3.1) and DAGEs (Section 3.3). Soluble hopanoids occur mostly in BD extracts, but there is a significant proportion (30%) of geohopanoids in Sox extracts of CP2. Insoluble hopanoids occur in all sinters (8–29%), but differ not only in

proportions but also in the partitioning between BHy and AMe fractions. Insoluble hopanoids in OP occur only in the BHy extracts (8%) and not in the AMe extracts. In contrast, the majority of insoluble hopanoids in CP2 occur in the AMe fraction (22% vs. just 7% in BHy extracts).

The distribution of geohopanoids in CP2 and OP differs among the OM fractions and also between the sinters (Fig. 3b). For example, the ratio of C_{31} vs. C_{32} hopanoic acid is lower – by a factor of 1.2 (CP2) and 1.6 (OP) – in the IOM fractions. This indicates that particular bacterial sources, those producing higher concentrations of C_{32} hopanoic acid precursors, might be more inherently insoluble or more prone to becoming so. Furthermore, the C_{31} isomerisation index, i.e. of the ratio of concentrations of C_{31} geohopanoids with ($\alpha\beta + \beta\alpha$) configurations vs. those with $\beta\beta$ configuration, is higher, by a factor of 1.4 (CP2) and 6.4 (OP), in the IOM fractions, indicating that the IOM is preferentially enriched in the mature configuration (Peters and Moldowan, 1991). No trend in the analogous C_{32} index is observed between soluble and IOM pools. These parameters also differ between the sinters (Fig. 3b), with C_{31} vs. C_{32} ratios ranging from 0.22 (OP) to 0.57 (CP2), and the C_{31} and C_{32} isomerisation indices ranging from 0.13 (CP2) to 3.2 (OP). This latter observation is consistent with accelerated maturation under high temperatures (Peters and Moldowan, 1991).

3.3. Bacterial DAGEs

Concentrations of DAGEs (VII) range from 0.81 (OP) to 5.7 mg g^{-1} TOC at CP1 (Fig. 4a), and no DAGEs were identified in CP2. In both the CP1 and OP sinters the vast majority of DAGEs are in the extractable fractions (84% and 99%, respectively), predominantly in the BD fraction. In the OP sinter, insoluble DAGEs occur in the BHy fraction as a minor fraction (1.4%). In contrast, in CP1 the insoluble DAGEs occur in both the BHy and AMe extracts, with ca. 8% of the lipids in each.

The average chain length (ACL) of the DAGEs differs among the two sinters (Fig. 4b; see also Kaur et al., 2015). The dominant DAGEs in CP1 contain alkyl moieties in combinations of C_{16}/C_{17}, C_{17}/C_{17} and C_{18}/C_{18}, resulting in an ACL of around 17.1. In the OP sinter there are higher abundances of DAGEs with C_{17}/C_{17}, C_{17}/C_{18} and C_{18}/C_{18} combined alkyl moieties, which results in slightly higher ACL values (ca. 17.5), consistent with the proposed adaptation of bacteria to increasing temperature

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(Paula et al., 1996; Sollich et al., 2017). However, there are no differences in DAGE ACL among soluble and IOM fractions – BD, Sox, BHy and AMe – in either sinter (Fig. 4b).

3.4. Archaeol

Concentrations of archaeol (VIII) range from 0.23 (CP2) to 0.63 mg g^{-1} TOC at CP1 (Fig. 5a). Similar to the bacterial DAGEs and hopanoids, most of the archaeol is soluble, predominantly in the BD fraction (up to 98%). Only 2% of the total archaeol occurs in OP insoluble fractions, all in the BHy extracts. In contrast, up to 22% of the total archaeol occurs in the CP insoluble fractions, in both the BHy and AMe fractions (CP1) or only in the BHy fractions (CP2). Ratios of archaeol vs. the bacterial analogue diethers – DAGEs – vary across all OM fractions and also among the different sinters (Fig. 5b), with ratios 1.3–2.0 times higher in IOM fractions, and up to seven times higher in the OP sinters as compared to CP1.

3.5. i-GDGTs

Concentrations of i-GDGTs (IX – XIV) range from 0.87 (CP2) to 5.4 mg g^{-1} TOC at OP (Fig. 6a). Similar to archaeol, the highest concentrations of i-GDGTs occur in the active sinters, CP1 and OP. However, the proportion of i-GDGTs occurring in non-extractable fractions are much higher than for archaeol, 43% in CP1, 21% in CP2 and 8% in the OP sample. In contrast to archaeol, which occurred preferentially in BHy extracts, insoluble i-GDGTs occur in both BHy and AMe fractions, almost equally (Fig. 6a). Distributions (i.e. degree of cyclisation) of i-GDGTs among the three sinters are different (Fig. 6b), but for a given sample they are similar in all four fractions – BD, Sox, BHy and AMe – consistent with the behaviour of DAGEs.

4. Discussion

Lipid biomarkers encountered in TVZ silica sinters have been comprehensively detailed previously (Kaur et al., 2011a; 2011b; 2015), and their sources tentatively attributed to a wide range of hyperthermophilic bacteria (e.g. the order Aquificales and some species such as *Ammonifex degensii*, *Thermodesulfobacterioum commune*, *Clostridium thermosulfurogenes*) and hyperthermophilic archaea (e.g. the orders of Sulfolobales, Thermoproteales and Thermoplastales) (Kaur et al., 2011a; 2011b; 2015; and references therein). Here, we focus on the comparison of prokaryotic membrane lipids

obtained from solvent-extraction, BD and Sox, vs. the analogues in IOM fractions, BHy and AMe. These findings are interpreted in the context of previous findings from a peat profile (Chaves Torres and Pancost, 2016) and coastal marine sediments (Chaves Torres et al., 2017), which were explored using the same methodology as in the current TVZ silica sinters. This allows us to explore the implications of insolubisation on the determination and interpretation of lipid biomarkers.

4.1. Soluble vs. IOM in TVZ silica sinters

Overall, there are some common observations amongst the three TVZ geothermal sinters. First, the proportion of prokaryotic biomarkers that are non-extractable with solvent-based techniques is significant, 14–45% of the branched FAs (Fig. 2), 6–29% of hopanoids (Fig. 3), 1–17% of DAGEs (Fig. 4), 1–22% of archaeol (Fig. 5) and 8–43% of i-GDGTs (Fig. 6). It is also highly variable, as reflected by those ranges, among the three samples measured here. With the exception of archaeol, which has not been observed previously in IOM of peat and marine sediments, these findings are consistent with previous work from profoundly different settings (Chaves Torres and Pancost, 2016; Chaves Torres et al., 2017).

Second, as observed in other settings, the partitioning among soluble and insoluble pools varies amongst lipid classes. Although the small number of samples dictates caution, some of these patterns occur in all three sinters. Ratios of i-GDGTs vs archaeol are up to 6 times higher in the IOM fractions than corresponding soluble ones (Fig. 5c), and ratios of archaeol vs. DAGEs are also higher in the IOM fractions (Fig. 5). These patterns exhibit intriguing similarities to previous work. The preferential occurrence in the IOM of archaeal ether lipids, both archaeol and i-GDGTs, over the bacterial DAGE analogues cannot be directly compared to previously studied peat and marine samples which lacked DAGEs. However, in those settings i-GDGTs are preferentially incorporated into IOM fractions relative to the bacterial branched GDGTs (br-GDGTs; Chaves Torres and Pancost, 2016; Chaves Torres et al., 2017), as was also observed in previous work (Tierney et al., 2012; Weijers et al., 2011). This could be due to a range of factors in the marine setting, where br-GDGTs were likely allochthonous (Zell et al., 2014), but the analogous observation in peat and now geothermal settings suggests that bacterial ether lipids are less prone to occurring in IOM fractions.

There are various ad hoc explanations that could account for the overall archaeal predominance in the IOM. For example, archaeal cells' particular adaptations to harsh environments (Valentine, 2007) – via for example, biosynthesis of particular IP head groups that reduce cell permeability (e.g. Curatolo, 1987; Baba et al., 2001; Shimada et al., 2008; Wang et al., 2012; Sollich et al., 2017) – could hinder solvent-extraction and/or increase archaeal cell preservation potential. In fact, the total lipid content of archaeal intact cells is typically underestimated with solvent extractions and requires chemical degradation treatments such as AMe (Koga et al., 1993; Koga and Morii, 2006).

Alternatively or additionally, the preferential occurrence of certain prokaryotic biomarkers in the IOM could arise from different diagenetic fates associated with different IP moieties. For example, archaeol – which occurred only in solvent extracts of peat (Chaves Torres and Pancost, 2016) and marine sediments (Chaves Torres et al., 2017) – typically contains phosphatidyl moieties (XV) (e.g. Sturt et al., 2004; Lipp and Hinrichs, 2009; Peterse et al., 2011), whereas i-GDGTs frequently occur as glycolipids (XVI) (Schouten et al, 2007; Liu et al., 2011; Lengger et al., 2012) and have been frequently reported in both soluble and IOM fractions (Huguet et al., 2010; Weijers et al., 2011; Tierney et al., 2012; Chaves Torres and Pancost, 2016; Chaves Torres et al., 2017). On the contrary, in hydrothermal environments archaeol has typically been reported not only as a phospholipid (XV)but also as a glycolipid (XVI) (Bradley et al., 2009), or only as a glycolipid (Gibson et al., 2013; Sollich et al., 2017), potentially an adaptation strategy in response to higher temperature or lower pH (Curatolo, 1987; Baba et al., 2001; Shimada et al., 2008; Wang et al., 2012; Sollich et al., 2017). It is intriguing then, that i-GDGTs occur in the IOM in all settings and archaeol occurs in IOM only in geothermal ones (to date). Previously, we tentatively invoked the relative lability of phosphatidyl moieties as an explanation for their poor capacity to facilitate insolubilisation of archaeol (Chaves Torres et al., 2017). Rapid loss of such moieties would preclude their role in diagenetic reactions leading to insolubilisation. Such explanations are tentative and require further examination, especially because: a) the difference of phospho- vs. glycolipid chemical lability might not be as large as traditionally thought (e.g. Logemann et al., 2011; Elling et al., 2017); and b) some archaeal lipids containing phosphatidyl IP moieties have been reported unrecovered even with AMe (Morii et al., 1986; Koga and Morii, 2006), which typically cleaves all glycosyl containing IP motifs. . Third, other compound

classes exhibit more diverse behaviour that is nonetheless consistent among the sinters. For example, the IOM of CP2 and OP is relatively enriched in C_{32} over C_{31} hopanoic acids, which could reflect different fates for different bacterial sources (Kaur et al., 2011a) with the former biological precursors more prone to insolubilisation. In contrast, other compound class characteristics, such as branched vs. straight-chain FAs, i-GDGT cyclisation and DAGE ACLs, are similar amongst all OM fractions, possibly indicating a uniformity of incorporation into the IOM. A final intriguing observation is that of the two active samples, CP1 and OP, the proportion of solvent non-extractable biomarker is markedly lower in the latter sinter, which was formed at higher temperatures (Figs. 2–6). Because this represents only two samples, this observation must be validated. If it is, the lower proportions of solvent non-extractable lipids in OP could be due to the loss of moieties critical to insolubilisation at higher temperature, consistent with the loss of functional groups at higher temperatures during OM diagenesis (Hatcher and Clifford, 1997).

4.2. Comparison of soluble and IOM fractions among diverse settings: insights into mechanisms

The current investigation of TVZ geothermal sinters and previous work on coastal marine sediments offshore Capetown (Chaves Torres et al., 2017) and on a *Sphagnum* peat (Chaves Torres and Pancost, 2016) reveal patterns that are both consistent and variable across multiple depositional regimes (Fig. 7). Across most compound classes, high proportions of lipids are released from the IOM after chemical degradation – BHy and/or AMe (Fig. 7). This occurs in every setting, with the percentage of prokaryotic biomarkers occurring in IOM fractions ranging from 1–45% in silica sinters, 5–80% in marine sediments and 5–65% in a *Sphagnum* peat bog (varying among samples but especially among different compound classes). Moreover, this high degree of association with IOM occurs at very early stages of diagenesis – less than 1ky in peat (Figs. 7o to 7s) and sinters (Figs. 7a to 7d) and a few millennia in marine samples (Figs. 7e to 7n). These observations are also in line with previous investigations where hopanoids (Farrimond et al., 2003) or GDGTs (Pancost et al., 2008; Huguet et al., 2010) have been identified in insoluble fractions of recent sediments or soils, interpreted as their rapid incorporation into IOM.

The large (1–80%) percentages of prokaryotic lipids in shallow peat and marine sediments and in recently deposited geothermal sinters is likely evidence of inherent insolubility of lipids and/or cells.

This was argued in previous papers, but similar observations in a setting where nearly all OM is prokaryotically derived reinforces that interpretation. Typically, inherent insolubility and recalcitrance are attributed to large biomacromolecules such as algaenans, cutans, suberans and lignin (Stach and Murchison, 1982; Largeau et al., 1984; 1986; Nip et al., 1986a; 1986b; Derenne et al., 1988; Nip et al., 1989; Tegelaar et al., 1989; 1995). However, previous work has also reported the inherent insolubility of lipids in prokaryotic cells (Philp and Calvin, 1976; Zelles, 1999; Sinninghe Damsté, 2014). In this case, the fact that IOM in recent sediments is enriched in prokaryotic membrane lipids likely indicates that prokaryotic cells can behave analogously to biomacromolecules such as lignin, algaenan and cutans, traditionally associated with the selective preservation pathway (e.g. Stach and Murchinson, 1982; Largeau et al., 1984; Derenne et al., 1988, Tegelaar et al., 1989). Hence, prokaryotic cells are partially insoluble to standard solvent-extractions, and further chemical treatment should be performed for a comprehensive lipid assessment.

An additional control on early OM preservation could be encapsulation in authigenic minerals (Schultze-Lam et al., 1995; Konhauser et al., 2003; Pancost et al., 2005). In marine sediments, up to 50% of the lipids occur in fractions obtained after dissolution of marine $CaCO_3$ with HCl_{aq} (Chaves Torres et al., 2017). Hence, it was proposed that interaction with carbonates (e.g. Ramseyer et al., 1997; and references therein), including physical encapsulation and sorptive interactions of lipids, is one of the main pathways for prokaryotic lipid insolubilisation. An analogous process could occur in silica sinters, especially because non-covalent OM-silica interactions have been previously reported (e.g. Davis, 1982; Parida et al., 2006; Nguyen and Chen, 2007; Kitadai et al., 2009; Bui and Choi, 2010), and in fact, result in microbial cells serving as nucleation surfaces for silica precipitation (Jones et al., 1997; Jones and Renaut, 2003; Handley et al., 2008). Although this has not been directly tested by dissolving the TVZ sinters, it is likely that such processes account for some of our observations. Both BHy and AMe are expected to induce a stronger swelling effect upon the silica matrix compared to exclusively organic solvent-extraction, for example, via disruption of electrostatic interactions (after e.g. Matturro et al., 1985; Agun et al., 2005) between silica (e.g. hydroxyl groups) and prokaryotic reactive interfaces for silica adsorption (Konhauser and Ferris, 1996; Konhauser et al., 2001) – e.g. via hydroxyl and carboxyl groups. Consequently, this swelling could facilitate solvent access to

encapsulated organics. Moreover, the higher temperature during the hydrolyses could facilitate desorption of organics from the silica matrix (Noller and Ritter, 1984). Thus, a proportion of the prokaryotic lipids encountered in BHy and AMe fractions might have been simply non-covalently released from the silica matrix, analogous to OM-carbonate dissociation proposed for the marine sediments (Chaves Torres et al., 2017). It is important to note that in the current TVZ study it is not possible to differentiate such released lipids from those that have been hydrolysed. However, the fact that the non-functionalised *n*-alkanes occur in both the extractable and IOM pools is likely evidence for encapsulation mechanisms, as has been previously proposed (Amblès et al., 1996; Chaves Torres et al., 2017). Curiously, *n*-alkanes occur in IOM fractions of marine sediments (Amblès et al., 1996; Chaves Torres et al., 2017), but not in peat (Chaves Torres and Pancost, 2016), suggesting that encapsulation of OM within authigenic minerals is important in IOM formation, consistent with previous work (Lalonde et al., 2012; Chaves Torres et al., 2017).

Collectively, the processes inferred to occur in the geothermal sinters of the current study as well as in a *Sphagnum* peat bog (Chaves Torres and Pancost, 2016) and in marine sediments (Chaves Torres et al., 2017) indicate that OM is being transferred into or occurs in IOM fractions even at early stages of diagenesis. Moreover, there is a preferential occurrence of archaeal lipids in IOM fractions in all settings, which implies an underestimation of the archaeal lipid composition when strictly solvent-extractable lipids are assessed. For example, the terrestrial input is likely overestimated when using the Branched and Isoprenoid Tetraether index (BIT) in marine sediments (Chaves Torres et al., 2017). The formation of the IOM might nevertheless be dynamic with time as manifested in decreasing IOM proportions with geological time in peat (Chaves Torres and Pancost, 2016), and also in the oldest TVZ sinter of the current study (CP2; Figs. 2, 5 and 6) and in previous investigations on marine sediments (Weijers et al., 2011). However, it is well established that overall insolubilisation of OM promotes OM persistence in the geosphere due to a decreased number of access sites for chemical and microbial degradation (Durand, 1980; Tissot and Welte 1984). Hence, the occurrence of a large proportion of IOM in TVZ silica sinters (Figs. 2 – 6) has not only direct implications for carbon bioavailability and climate, but also for astrobiology studies (Walter and Des Marais, 1993; Ruff and Farmer, 2016) and early Earth origin of life investigations (Konhauser et al., 2003), where the IOM

fractions are expected to comprise the OM with the highest preservation potential. Finally, the IOM released in the current study – despite being an important repository for specific lipid classes – is only a small fraction of the TOC (<3.5% see Supporting information Table S5) and the IOM of the TVZ silica sinters. Further and harsher selective chemical degradation steps or even pyrolysis could be performed upon our final residues (R4AMe), allowing of the remaining IOM (see reviews e.g. Rullkötter and Michaelis, 1990; Vandenbroucke and Largeau, 2007) and a better understanding of the markers for life that it contains.

5. Conclusion

Prokaryotic lipids in TVZ sinters do occur in insoluble fractions, in significant but highly variable amounts, i.e. as low as 1% of the DAGEs occur as IOM and as much as 45% of the FAs, with this likely being minimum yields). In general, patterns of formation/occurrence of IOM are the same as observed in previous studies, but some exceptions do occur. For example, archaeol occurs in TVZ sinter IOM fractions, both BHy and AMe, in contrast with peat and marine sediments. This indicates that IOM formation in TVZ silica sinters differs from those other settings, possibly due to distinctive lipid IP moieties of the extremophiles or to different OM insolubilisation processes. The fact that IOM occurs in recently (collectively <10 y to <5,000 y) formed sediments or deposited sinters likely reflects inherent insolubility of prokaryotic cells. Although further work is needed, we also propose that the OM-silica association in silica sinters, analogous to the OM-carbonate association inferred in marine sediments, facilitates OM preservation. Crucially, these results extend previous findings that certain lipids are more prone to occur in IOM and hence, to be preserved with geological time, i.e. i-GDGTs and archaeol relative to analogous bacterial ether lipids. Further work is needed to explore the scope and mechanisms underlying these observations. However, the fact that large proportions of prokaryotic lipids occur predominantly in IOM fractions and furthermore, that archaeal lipids are more likely to be insoluble, suggests the importance of IOM analysis to avoid potential bias in early Earth and astrobiology studies and to maximise lipid recovery.

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Figure 1. Partial chromatograms of BD (AMe of TLE), Sox (AMe of TLE), BHy and AMe OM fractions from a Champagne Pool silica sinter (CP1) after GC-MS. Symbols: black circle: alkanoic acids; β: β-hydroxy alkanoic acids; ω: ω-hydroxy alkanoic acids; a: alcohols; P: polycyclic aromatic hydrocarbons (PAHs); d: dialkyl glycerol diethers (DAGEs); s: sterols; A: archaeol; Ph: phenols; is: internal standard (2-hexadecanol).

Figure 2. (a) Percentage relative abundances of total branched FAs concentrations (*iso-* and *anteiso-* C_{15} and C_{17} FAs) among OM fractions. Numbers within pies represent concentrations (mg g^{-1} TOC) of branched FAs in each OM fraction. (b) Ratio total branched (*iso-* and *anteiso-* C_{15} and C_{17} FAs) vs. total straight-chain (*n-*C_{15} and n-C_{17}) FAs in soluble and IOM fractions.

Figure 3. (a) Percentage relative abundances of total hopanoids obtained after BD, Sox, BHy and AMe. Numbers within pie charts represent concentrations of hopanoids (mg g^{-1} TOC). (b) Distributions of hopanoids in CP2 (top) and OP (bottom).

Figure 4. (a) Percentage relative abundances of total bacterial DAGEs identified in BD, Sox, BHy and AMe fractions. Numbers within pies represent concentrations (mg g^{-1} TOC). (b) Average chain length (ACL) of DAGEs that occur in CP1 and OP.

Figure 5. (a) Percentage relative abundances of archaeol identified in BD, Sox, BHy and AMe fractions. Numbers within pies indicate concentrations of archaeol (mg g^{-1} TOC). (b) Ratio of concentrations of archaeol vs. total concentrations of bacterial DAGEs. (c) Ratio of concentrations of archaeol vs. total concentrations of i-GDGTs.

Figure 6. (a) Percentage relative abundances of i-GDGTs identified in BD, Sox, BHy and AMe fractions of TVZ silica sinters. Numbers within pies represent concentrations of i-GDGTs (mg g^{-1} TOC). (b) Distributions of i-GDGTs that occur in CP1 (top), CP2 (middle) and OP (bottom). Note apparent differences among samples, but similarities among OM fractions.

Figure 7. Percentage relative abundances of extractable (BD and Sox) and insoluble (BHy and AMe) prokaryotic biomarkers from TVZ silica sinters (left), Eastern South Atlantic Ocean (ESAO)

continental slope sediments (middle; after Chaves Torres et al., 2017) and a UK *Sphagnum* peat bog (right; after Chaves Torres and Pancost, 2016).