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Bacterial and archaeal lipids trace chemo(auto)trophy along the redoxcline in Vancouver Island fjords

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Abstract

Marine oxygen minimum zones play a crucial role in the global oceanic carbon, nitrogen, and sulfur cycles as they harbor microbial communities that are adapted to the water column chemistry and redox zonation, and in turn control the water column chemistry and greenhouse gas release. These micro-organisms have metabolisms that rely on terminal electron acceptors other than O_2 and often benefit from syntrophic relationships (metabolic coupling). Here, we study chemo(auto)trophy along the redoxcline in two stratified fjords on Vancouver Island (Canada) using bacterial bacteriohopanepolyols and archaeal ether lipids. We analyze the distribution of these lipid classes in suspended particulate matter (SPM) to trace ammonia oxidation, anaerobic ammonium oxidation (anammox), sulfate reduction/sulfur oxidation, methanogenesis, and methane oxidation, and investigate ecological niches to evaluate potential links between their respective bacterial and archaeal sources. Our results show an unparalleled BHP and ether lipid structural diversity that allows tracing the major redox-driven metabolic processes at the time of sampling: Both fjords are dominated by archaeal ammonia oxidation and anammox; sulfate-reducing bacteria may be present in Deer Bay, but absent from Effingham Inlet; methanogenic Euryarchaeota and archaeal and bacterial methanotrophs are detectable at low abundance. Correlation analysis reveals distinct biomarker clusters that provide constraints on the biogeochemical niches of some orphan BHP and ether lipids such as in situ-produced adenosyl-BHPs or unsaturated archaeols.

KEYWORDS
archaeal ether lipids, bacteriohopanepolyols, chemo(auto)trophy, redox gradient, Vancouver Island fjord, water column suspended particulate matter

1 | INTRODUCTION

Marine oxygen minimum zones (OMZs) have received increasing attention given their capacity to release greenhouse gases, control on biogeochemical cycles, and spatial expansion over the past decades (Keeling & Garcia, 2002; Keeling et al., 2010; Naqvi et al., 2010; Schmidtko et al., 2017). Greenhouse gas emissions from OMZs are caused by microbial respiration occurring along strong redox gradients that establish in the absence or reduction in water column ventilation. At decreasing and ultimately vanishing O_2 concentrations

along the oxycline, these heterotrophic and chemo(litho)autotrophic organisms derive their energy from metabolisms relying on other terminal electron acceptors than O_2, such as NO_3^-, Mn, Fe, SO_4^{2-}, and CO_2 (Ulloa et al., 2012; Wright et al., 2012). The most prominent metabolic processes in OMZs include bacterial and archaeal ammonia oxidation, bacterial denitrification and anaerobic ammonium oxidation (anammox), bacterial sulfur oxidation and sulfate reduction, and archaeal and bacterial methane oxidation (Kuypers et al., 2005; Lam et al., 2009; Lavik et al., 2009; Wakeham et al., 2003). Accordingly, OMZs play a key role in the coupled oceanic carbon, nitrogen, and sulfur cycles (Wakeham, 2020; Wright et al., 2012). In addition to identifying metabolisms and quantifying process rates in OMZs, the focus in recent years has been extended to include a better understanding of metabolic coupling within the diverse microbial community, that is, the biogeochemical co-dependence of syntrophic community networks with energy flow via metabolite exchange (Hawley et al., 2014; Hawley et al., 2017; Wright et al., 2012).

Lipid biomarkers constitute a more quantitative, although less specific, approach to trace chemoautotrophy in OMZ settings compared to genomic tools (e.g., Cantarero et al., 2020; Wakeham, 2020). For example, Wakeham et al., (2007) demonstrated how the distribution of archaeal, bacterial, and eukaryotic biomarkers mirrors the complex chemistry and microbial ecology within the chemocline in the Black Sea. In a subsequent investigation, Wakeham et al., (2012) studied the biomarker distribution along redox gradients in the Cariaco Basin and showed that diagnostic biomarkers allow tracing the major biogeochemical processes. Similarly, a recent study by Cantarero et al., (2020) showed that the distribution of intact polar lipids (IPLs) in the OMZ of the Eastern Tropical South Pacific exhibits unique signatures that are characteristic of different biogeochemical niches in the water column. Given their degree of environmental and taxonomic specificity (examples outlined below), archaeal glycerol dialkyl glycerol tetraethers (GDGTs) and diethers (archaeols, ARs), as well as bacterial bacteriohopanepolyols (BHPs), are particularly useful biomarkers to trace archaea and bacteria; however, BHPs are not produced by all bacteria and the origin of many ether lipids and BHPs can be multi-modal. Archaeal lipids can be found either as IPLs (consisting of apolar core lipids (CLs) linked to polar head groups) or free CLs (derived from hydrolytic cleavage of polar headgroups of IPLs). For instance, the core GDGT crenarchaeol and IPLs containing crenarchaeol (Figure S1) have thus far only been detected in ammonia-oxidizing (Marine Group I, MG I) Thaumarchaeota (Schouten et al., 2013). Furthermore, the phylogenetic subgroups within this phylum produce characteristic ether lipid distributions (Elling et al., 2017) that also differ from those in methanogenic and methanotrophic Euryarchaeota (Schouten et al., 2013). These differences allow differentiating ether lipid source organisms in environmental samples. Some BHPs are equally diagnostic. An isomer of bacteriohopanetetrol (BHT), which is common in OMZs (Matys et al., 2017; Sáenz et al., 2011), has been identified as a biomarker for anammox based on its presence in marine "*Ca*. Scalindua profunda" and "*Ca*. Scalindua brodeae" enrichments as well as freshwater "*Ca*. Kuenenia stuttgartiensis" and "*Ca*. Brocadia spp." enrichment cultures (Rush

et al., 2014; Schwartz Narbonne et al., 2020) in addition to its δ^{13}C-depleted signatures in cultures and the environment (Hemingway et al., 2018; Lengger et al., 2019). However, trace amounts of BHT isomer have also been found in oxic marine environments (Kusch et al., 2018, 2019; Matys et al., 2017). This conundrum has now been resolved by a new GC method that revealed the presence of two BHT isomers (BHT-II and BHT-x) that otherwise co-elute during standard HPLC analysis, of which BHT-x is now considered to be the more robust anammox biomarker (Schwartz Narbonne et al., 2020). Other BHPs such as methylcarbamate-substituted amino-BHPs and aminopentol have primarily been identified in methane-oxidizing bacteria (Rush et al., 2016) and can, thus, be linked to aerobic methanotrophy. Trace amounts of aminopentol and aminotetrol have been identified in sulfate-reducing bacteria (Blumenberg et al., 2006, 2009, 2012), and their amount relative to aminotriol may allow tracking this important anaerobic process (Rush et al., 2016; Talbot et al., 2016). Therefore, the combined analysis of ether lipids and BHPs provides an important tool to infer chemo(auto)trophic processes along strong oceanic redox gradients. This multiple biomarker class approach should also provide insights regarding the ecological niche and potential bacterial producers of adenosyl-BHPs (adenosylhopane and its related structures adenosylhopane-type 2 and adenosylhopane-type 3 with an unresolved adenine-like moiety as well as their C-2 methylated homologs; Figure S1). Kusch et al., (2021) have recently shown that these "soil marker" BHPs (Zhu et al., 2011) might have a chemotrophic origin in OMZ settings rather than being primarily supplied from the continent.

In this study, we investigate the BHP and ether lipid distributions in water column profiles of two stratified fjords on Vancouver Island to trace major redox-driven chemo(auto)trophic processes (ammonia oxidation, anammox, sulfate reduction/sulfur oxidation, methanogenesis, and methane oxidation) and to evaluate co-occurrence and/or niche partitioning between archaeal ether lipids and bacterial BHPs, which aids at constraining the sources of some ubiquitous/orphan lipids.

2 | STUDY AREA

The western coast of Vancouver Island, British Columbia, is characterized by narrow fjords nestled into the Vancouver Island Ranges. These estuarine-type fjords are connected to the Pacific Ocean, but water exchange is typically restricted by shallow sills (Pickard, 1963). Accordingly, water masses in many of these fjords, including Effingham Inlet and Tofino Inlet (Figure 1), show salinity-driven stratification, which along with productivity-driven respiration causes the occurrence of suboxic or anoxic bottom waters (Dallimore & Jmieff, 2010; Patterson et al., 2000; Pickard, 1963). The bathymetry of Effingham Inlet is characterized by outer and inner basins separated by a sill at ca. 40 m water depth. In the inner basin, the maximum water depth is approximately 120 m (Nuwer & Keil, 2005; Patterson et al., 2000) and anoxic conditions typically prevail below 60–80 m water depth, with both the

FIGURE 1 (a) Map of Vancouver Island and fjord locations discussed in the text. (b) Sampling stations in Deer Bay (Tofino Inlet) and Effingham Inlet
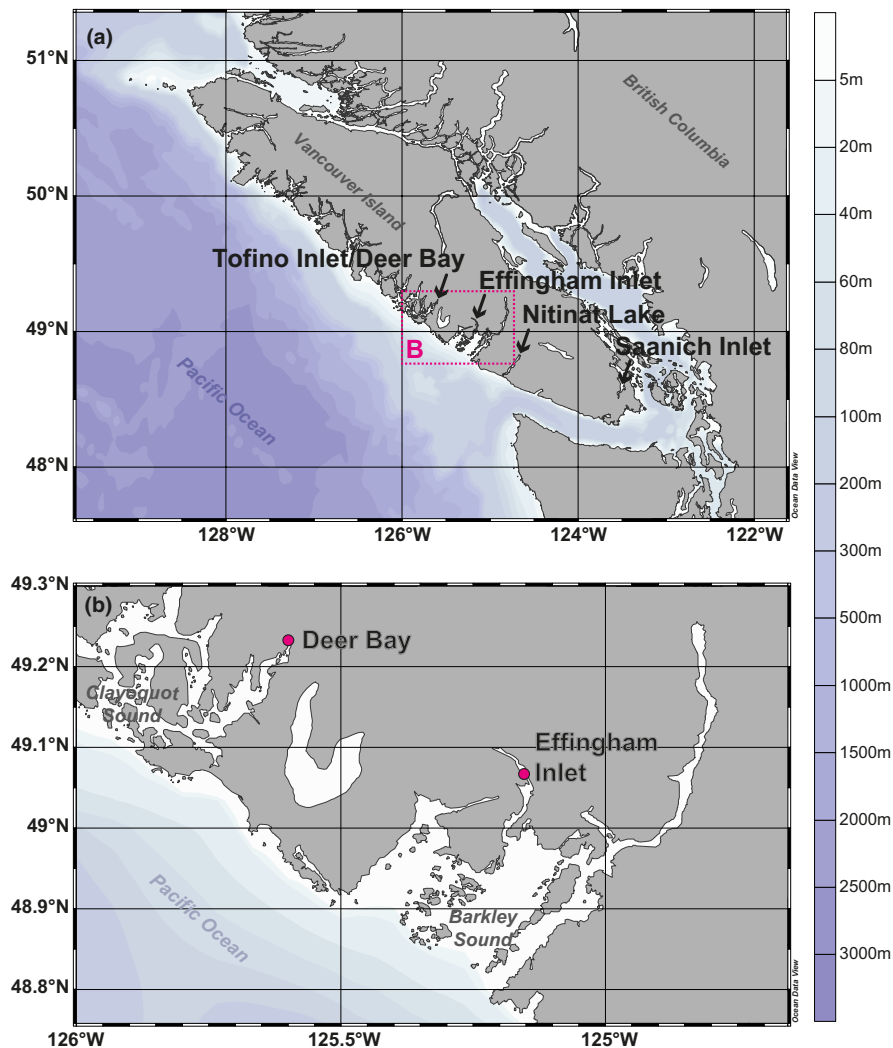
pycnocline and the redoxcline migrating upwards seasonally during the phytoplankton bloom (Diaz et al., 2012). At intermediate depths, within the halocline/pycnocline, waters are exchanged daily through tidal waves. In contrast, renewal of bottom waters only occurs roughly every other year once the flow of the Vancouver Island Coastal Current and the estuarine circulation in Barkley Sound are reversed, allowing upwelled shelf waters to intrude into Effingham Inlet (Thomson et al., 2017). Despite these ventilation events, anoxic bottom water conditions are typically restored after 1–2 months (Thomson et al., 2017). The bathymetry of Tofino Inlet is similar to the inner basin of Effingham Inlet, but it has an additional small basin at its head, Deer Bay, that is separated further by a sill at ca. 20 m water depth and has a maximum water depth of 40 m. In general, however, the water circulation in Tofino Inlet is essentially restricted through Browning Passage, its connection to Clayoquot Sound, which is characterized by waters as shallow as ~11 m.

Both, the inner basin of Effingham Inlet and Deer Bay are fed by small rivers, the Effingham River and Tofino Creek, respectively, and highest freshwater input occurs during the winter precipitation maximum (Dallimore & Jmieff, 2010; Nuwer & Keil, 2005). Runoff

during rainstorm events is the primary delivery mode of terrestrial organic matter (OM) from the temperate rainforests (Dallimore & Jmieff, 2010).

3 | MATERIALS AND METHODS

Suspended particulate matter (SPM) samples were collected in inner Effingham Inlet (lat 49.07, long −125.15) and in Deer Bay (Tofino Inlet; lat 49.22, long −125.60) on Vancouver Island (Figure 1b) onboard the *R/V Barnes* in July 2007. Particulate matter from approximately 72–275 L of water were collected using standard-sized McLane WTS-LV in situ filtration pumps; sampling depths were based on onboard CTD data (temperature, salinity, fluorescence, transmissivity, O_2; SEASOFT software, Seabird Electronics, Inc.) taken immediately before and after the pumping. All pump deployments at each site were within a 12-hr period (see Diaz et al., (2012) for replicate profiles in Effingham Inlet) and the sea-state was calm, which facilitated careful depth control within the constraints imposed by the sampling procedure and equipment (including ship motion). SPM was collected

from the chlorophyll maximum in surface waters (5–6 m water depth), at several depths across the respective redox transition of both inlets (72, 75, and 77 m water depth in Effingham Inlet and 23, 25, and 28 m water depth in Deer Bay), and from fully anoxic near-bottom waters (90 and 35 m, respectively). SPM was filtered onto pre-combusted, doubled 142 mm Whatman GF/F filters of 0.7 μm nominal pore size. Filters were immediately frozen and stored frozen until extraction back in the Skidaway laboratory. Total lipids were extracted using Soxhlet extractors with methylene chloride:methanol (2:1, v:v). As previously noted by Schubotz et al., (2018), Soxhlet extraction rather than Bligh & Dyer was the only feasible way to extract doubled GF/F filters, and no obvious bias was evident for ether lipids (Schubotz et al., 2018) or BHPs (Kusch et al., 2019, 2021) using this technique. Total lipid extracts (TLEs) were stored at −20°C until analysis.

A split of the TLE was used for BHP analysis. Prior to LC-MS analysis, 100 ng of the internal standard 5α-pregnane-3β,20β-diol was added to the samples, which were then acetylated using pyridine: acetic acid (1:1; v:v) at 50°C for 1 hr, and then left to react overnight at room temperature. The acetylated samples were filtered using 0.45 μm PTFE filters with methanol: propan-2-ol (3:2; v:v). BHPs were analyzed in the Organic Geochemistry Laboratory at the University of Colorado Boulder using a Thermo Ultimate 3000 HPLC – Q Exactive Focus Orbitrap MS system with APCI ion source operated using the chromatographic and MS conditions described by Kusch et al., (2019). BHPs were quantified against pregnane-diacetate without correction for differences in response factor due to the absence of authentic BHP standards. Accordingly, BHP concentrations are semi-quantitative, yet are directly comparable between all samples analyzed here. Adenosyl-BHP and BHT abundances in Effingham Inlet and Deer Bay were previously reported by Kusch et al., (2021).

Another split of the TLE was taken for archaeal biomarker analysis, and the methodology and data were previously published in Zhu et al., (2016). Ether lipids were analyzed in the Organic Geochemistry Laboratory at MARUM, University of Bremen using a Dionex Ultimate 3000 UHPLC with ESI ion source and coupled to a Bruker maXis qTOF system. Prior to analysis, instrument conditions were optimized using ether lipid standards of ß-L-gulosyl-phosphoglyceroldibiphytanyl glycerol tetraether (obtained from Matreya, LLC), phosphoethanolaminearchaeol (obtained from Avanti Polar Lipids Inc.), and GDGT-0 (isolated from a culture of *Archaeoglobus fulgidus*). Chromatographic separation, identification, and quantification of ether lipids were achieved using the method described by Zhu et al., (2013).

We report BHP and ether lipid abundances normalized to water volume since particulate organic carbon (POC) was only determined for the Effingham Inlet samples. For Effingham Inlet, we observe significant correlations between volume-normalized and POC-normalized BHP ($r = .986$; $p < .0001$) and ether lipid ($r = .948$; $p < .0001$) concentrations, that is, the depth trends do not stem from POC distributions in the water column. The POC-normalized data for Effingham Inlet are included in Tables S1 and S2.

# 4 | RESULTS

4.1 | Water column hydrography and chemistry

In this study, we define the water column as dysoxic at 20–90 μM O_2, suboxic at 1–20 μM O_2, and anoxic below 1 μM O_2 (to account for the sensor detection limit), although the boundaries between these zones are in reality gradients, that vary temporally. During sampling, Effingham Inlet was characterized by overall low O_2 concentrations throughout the water column. The highest O_2 concentrations in surface waters (4 m) were ~137 μM (Figure 2a), dysoxic conditions (<90 μM) were detected at 9 m depth, and O_2 levels became suboxic (<20 μM) at 56 m water depth. Anoxic conditions (<1 μM O_2) occurred below 78 m water depth. The SPM samples, thus, represent oxic (5 m), suboxic (72, 75, and 77 m), and anoxic (90 m) conditions. Fluorescence (Figure 2c) was highest in surface waters and decreased quickly within the uppermost 30 m of the water column. Transmissivity (Figure 2b) mirrored the fluorescence trend but showed an additional decrease between ca. 78 and 88 m water depth, which likely corresponds to the deep particle maximum at the chemocline.

In Deer Bay, surface waters were dysoxic with highest O_2 concentrations reaching 85 μM at 3 m depth and concentrations <20 μM below ca. 29 m water depth (Figure 2g). Anoxic conditions were detected below 33 m water depth. According to the CTD cast, all SPM samples except the 35 m sample (anoxic) would represent hypoxic conditions within the redox transition. However, the gradient from hypoxic to suboxic conditions occurs within a meter at 28–29 m depth; thus, within the constraints imposed by the sampling procedure and equipment (including ship motion), we consider the 28 m sample to be representative of suboxic conditions. Fluorescence (Figure 2i) was highest in surface waters and decreased below 16 m water depth. In contrast to Effingham Inlet, the Deer Bay water column showed sharper salinity-driven density stratification and revealed distinctly warmer bottom waters (Figure 2g).

Unfortunately, the original sampling operation in Effingham Inlet or Deer Bay did not include nutrient analysis.

4.2 | Bacteriohopanepolyols

In Effingham Inlet, total BHP concentrations were lowest in the oxic surface sample (ca. 0.3 μg/L) and highest in the anoxic sample (ca. 25.7 μg/L) (Figure 2c), with a slight concentration minimum at 75 m in the deep suboxic layer. In Deer Bay, total BHP concentrations were also low in the surface sample (ca. 2.7 μg/L) and highest in the anoxic sample (ca. 16.1 μg/L), and the lowest concentrations were detected at 25 m depth (ca. 2.4 μg/L). Both profiles, thus, showed an inversion of total BHP concentrations across the redox transition, but the inversion was much more pronounced in Deer Bay (Figure 2i). Despite the lowest total BHP concentrations, the samples within the redoxcline (75 m in Effingham Inlet
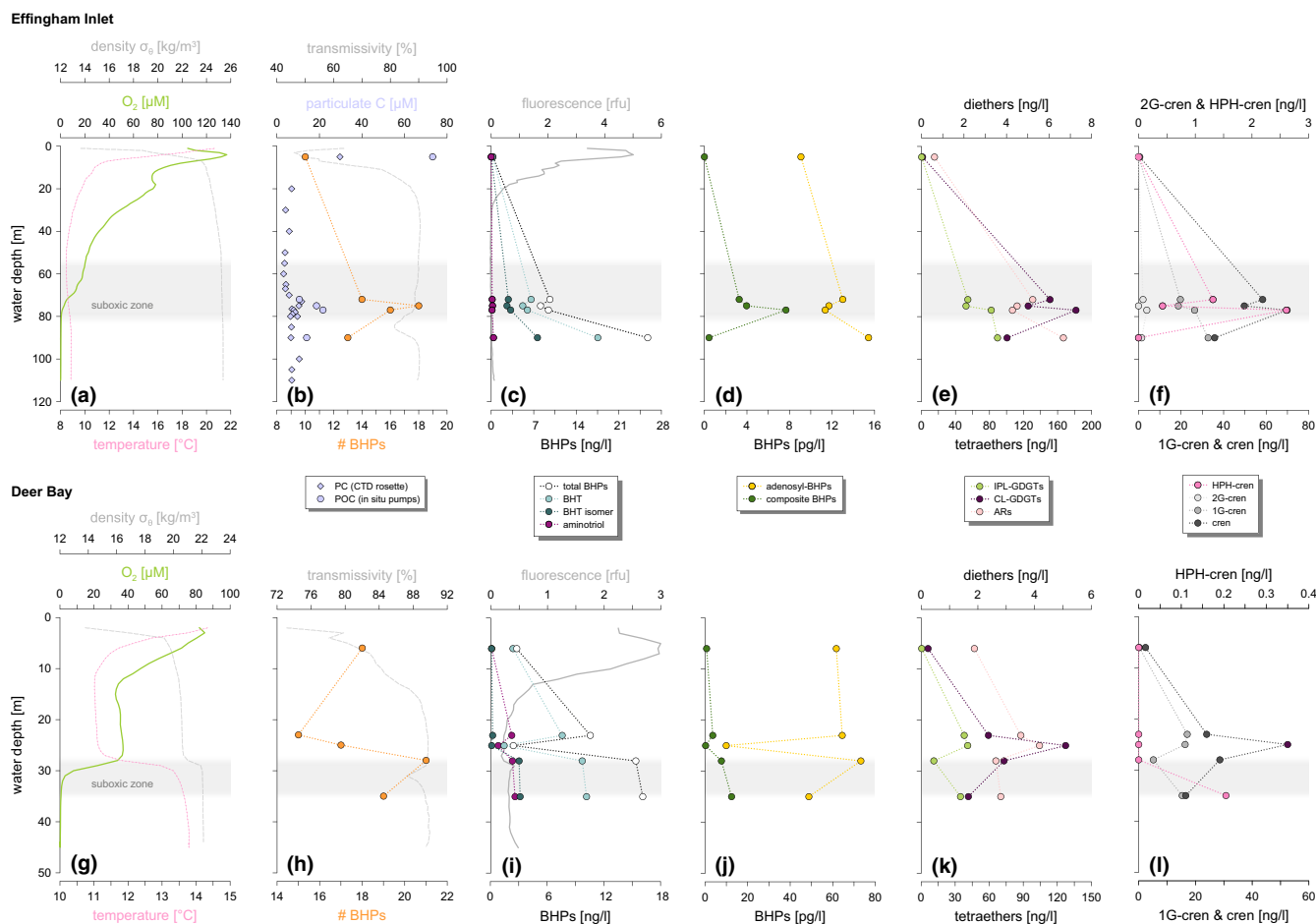
FIGURE 2 Hydrographic properties and biomarker distributions in the Effingham Inlet and Deer Bay water column. (a) and (g) Density, temperature, and O₂ concentrations; (b) and (h) transmissivity, particulate carbon (PC) and particulate organic carbon (POC) [from Diaz et al. (2012)], and BHP diversity (number of individual structures); (c) and (i) fluorescence (relative fluorescence units) and absolute abundance of total BHPs (ng/L) and the three most abundant individual BHPs: BHT, BHT isomer, and aminotriol (ng/L); (d) and (j) absolute abundance of adenosyl-BHPs and composite BHPs (pg/L); (e) and (k) absolute abundance of diether (ARs) and tetraether lipids (IPL-GDGTs and CL-GDGTs) (ng/L); (f) and (l) absolute abundance of crenarchaeol (cren) ether lipids (ng/L). Zero values in (c–f) and (i–l) indicate that individual compounds were not detected

and 25 m in Deer Bay) were characterized by high BHP diversity (18 and 17 structures, respectively; Figure S1) and, in fact, BHP diversity at 75 m depth was the highest in the Effingham water column (Figure 2b). The high BHP diversity in Effingham corresponds to a peak in particulate (organic) carbon and reduced transmissivity (Figure 2b), but BHP diversity and water volume (r = 0.349; p = .323) or POC (r = −0.691; p = .196) show no significant correlation. Overall, the BHP diversity was higher in Deer Bay (15–21 structures; Figure S1) in comparison with Effingham Inlet (10–18 structures), and aminopentol, aminotriol isomer, and 2Me-adenosylhopane were detected only in Deer Bay (Figure 2b,h; Table S1).

BHT, BHT isomer (co-eluting BHT-II and BHT-x), and aminotriol were the most abundant BHPs in all samples (Figures 3 and S2). In the Effingham water column, these BHPs accounted for roughly 93.4%–99.2% of total BHPs, although aminotriol accounted for only 0.7%–2.6% of total BHPs. In contrast, aminotriol represented 3.4%–33.0% of total BHPs in Deer Bay. In both inlets, BHT

isomer and aminotriol relative abundances generally increased with increasing water depth at the expense of BHT and all other BHPs, with the latter occurring well below 1% abundance, except for some adenosyl-BHPs in surface waters (Figure 3). The minor BHP assemblages were primarily composed of amino-BHPs and adenosyl-BHPs as well as other ubiquitous hydroxylated/cyclic BHPs (Figure 3), and their absolute abundances (Table S1) were higher in Deer Bay (up to ~560 pg/L) in comparison with Effingham Inlet (up to ~220 pg/L). The relative abundance of adenosyl-BHPs was highest in oxic surface waters (3.2% and 2.2% in Effingham Inlet and Deer Bay, respectively) and lowest in anoxic bottom waters (0.1% and 0.3%, respectively) at both locations, whereas amino-BHPs such as aminotetrol (up to 0.4%) and aminopentol (<0.1%) showed highest abundances in the redoxcline (Figure 3). In both inlets, composite BHPs were detected at low abundance (highest concentration of 12.4 pg/L at 35 m depth in Deer Bay) and some composite BHPs occurred almost exclusively at O₂ levels <10 μM (Table S1).
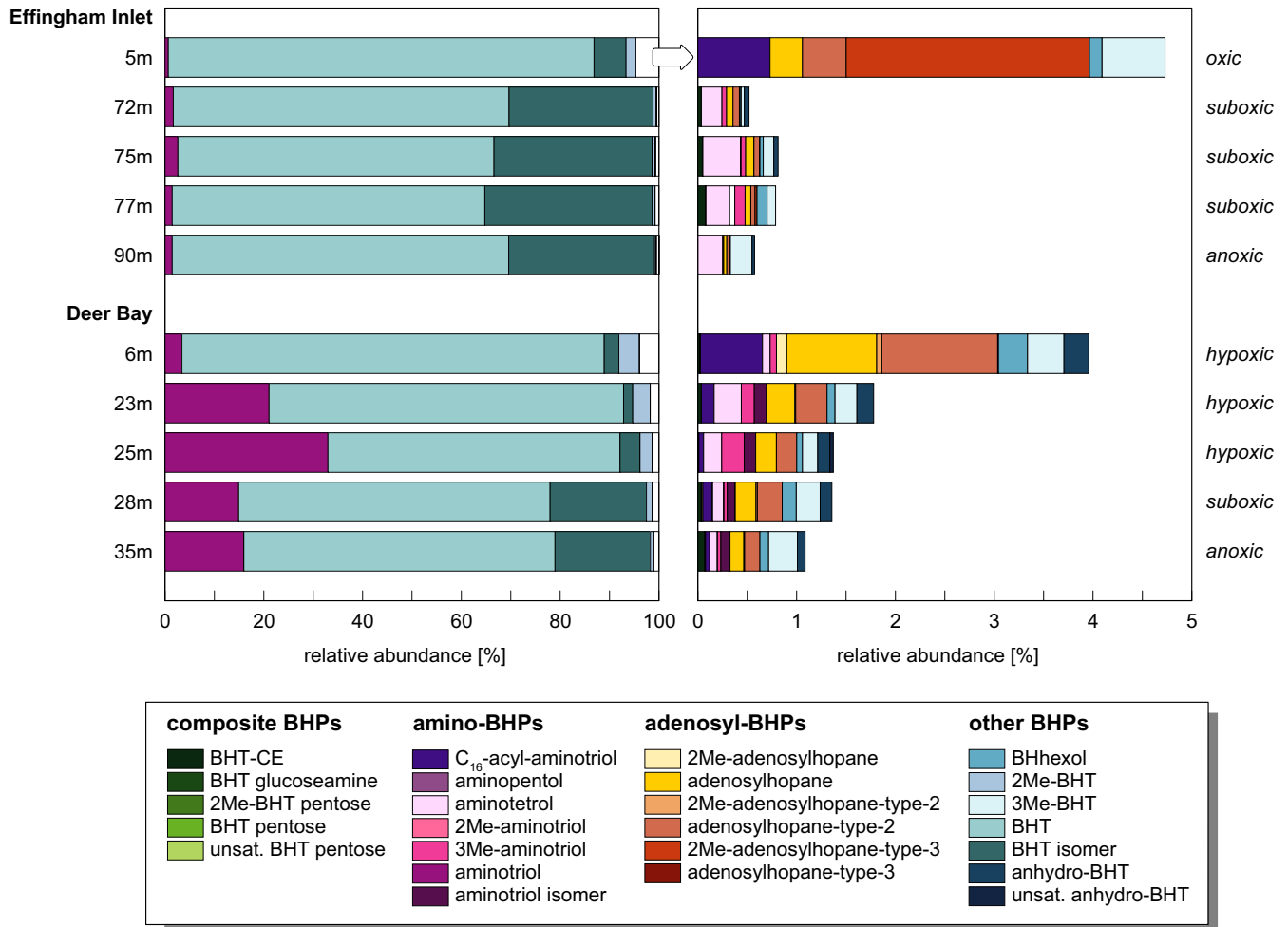
FIGURE 3 Relative abundance of major (left) and minor (right) BHPs in the Effingham Inlet and Deer Bay water column

4.3 | Archaeal ether lipids

Archaeal ether lipid data for Effingham Inlet and Deer Bay were previously published in Zhu et al., (2016) as part of an investigation of effects of environmental adaptation on the archaeal lipidome in diverse oceanic regions. Here, we examine the Effingham Inlet and Deer Bay dataset more thoroughly in terms of microbial metabolic processes. Total archaeal ether lipid concentrations (sum of all IPLs and CLs) were lowest (2.2–8.5 ng/L) in surface waters in both fjords (Figure 2e,j) and highest at 77 m depth in Effingham Inlet (ca. 270 ng/L) and at 25 m depth in Deer Bay (ca. 170 ng/L). IPLs, core GDGTs, and core ARs showed distinctly different patterns with depth in each fjord. In Effingham Inlet, IPL concentrations showed an almost linear increase with depth (to up to ca. 90 ng/L), whereas core GDGTs showed highest concentrations at 77 m (ca. 180 ng/L) and decreased below. Archaeols, on the other hand, peaked at 90 m depth (ca. 7 ng/L) and showed a brief inversion between 72 and 77 m water depth. In Deer Bay, IPLs (ca. 41 ng/L), core GDGTs (ca. 130 ng/L), and core ARs (ca. 4 ng/L) all peaked at 25 m water depth just above the suboxic boundary where BHPs showed a pronounced decrease. While core GDGTs decreased

toward anoxic bottom waters, both IPLs and ARs slightly increased in absolute abundance.

Overall, the distribution of archaeal lipids was relatively similar in both Effingham Inlet and Deer Bay (Figures 4 and S2). Oxic surface waters in Deer Bay contained only intact 1G-GDGTs (monoglycosyl-GDGTs), core GDGTs, core ARs, and core OH-GDGTs (hydroxy-GDGTs). ARs were the second most abundant ether lipid group in surface waters (28.2% and 22.2%, respectively) but decreased significantly (<4%) across the redoxcline. IPL relative abundances and diversity were lowest in surface waters (11.2% and 5.8%, respectively) and highest in the anoxic water masses (45.5% and 43.8%, respectively) in both Effingham Inlet and Deer Bay. With the exception of HPH-GDGTs (hexose-phosphohexose-GDGTs), only glycosidic IPLs were detected. The increase with depth in IPLs is consistent throughout the Effingham water column, whereas their abundances showed an inversion at 23 m and 25 m water depth in Deer Bay where CL abundances increased. Overall, the diversity (6–35 structures; Figure S1) and distribution of dialkyl structures were similar in Effingham Inlet and Deer Bay (Figure 5), suggesting a relatively similar archaeal community composition throughout the water column.
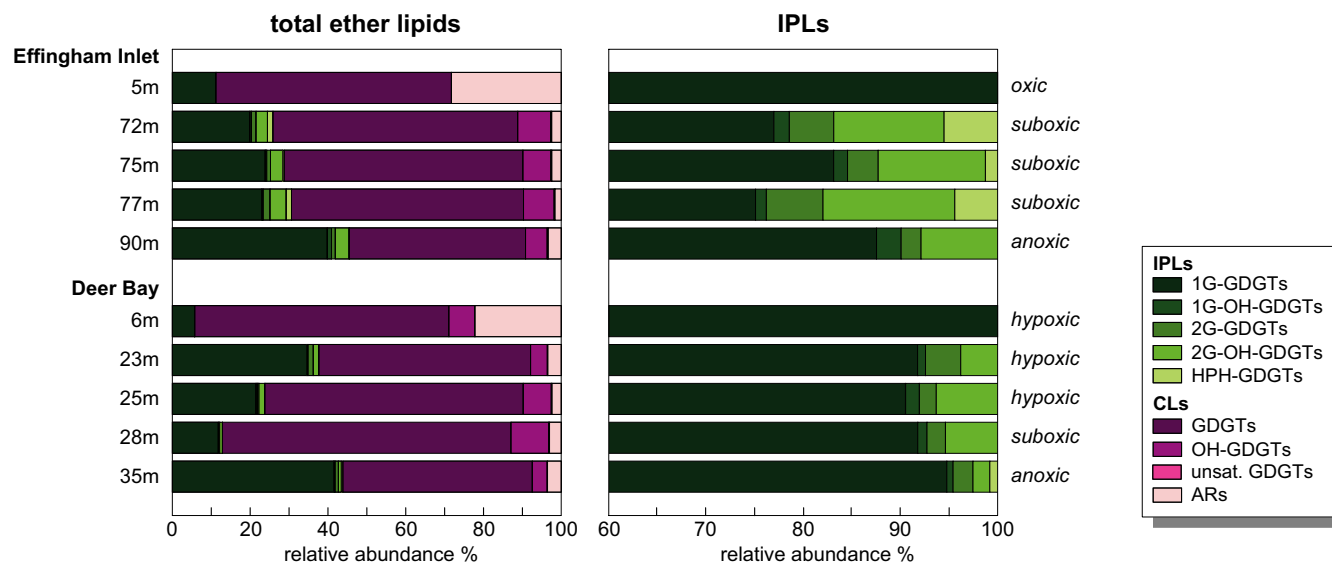
FIGURE 4 Relative abundance of total (intact and core) ether lipids (left) and intact (right) ether lipid classes detected in the Effingham Inlet and Deer Bay water columns. Please note x-axis scales

5 | DISCUSSION

We aim at evaluating the proficiency of archaeal ether lipid and bacterial BHP distributions to infer major chemo(auto)trophic processes along redox gradients including ammonia oxidation, anammox, sulfur reduction/oxidation, methanogenesis, and methanotrophy, and to evaluate potential links between lipids and their sources in two coastal fjords on Vancouver Island. We assume that the majority of ether lipids and BHPs represent in situ autotrophic or heterotrophic production, and that they reflect the stratification of the water column and the availability of terminal electron acceptors (within the constraints of sampling resolution, see Section 4.1). We expect the majority of lipids to derive from viable biomass and also exclude significant supply from the catchment. For BHPs, although both Effingham Inlet and Deer Bay are located in close proximity to land (Figure 1), Kusch et al., (2021) concluded that the majority of adenosyl-BHPs are produced in situ, and that only 2Me-adenosylhopane, adenosylhopane-type 3, and 2Me-adenosylhopane-type 3 may also be supplied from catchment soils, since they occur almost exclusively in surface, low-salinity waters. However, even if all adenosyl-BHPs in surface waters (where they represent 2.2% to 3.2% of total BHPs; Figure 3) were indeed supplied from the catchment, R_{soil} index values (a proposed proxy for soil input; Zhu et al., 2011) are 0.04 in Effingham Inlet and 0.03 in Deer Bay, indicating negligible soil contributions (Kusch et al., 2021). Likewise, our data suggest negligible supply of ether lipids from catchment soils. We find low ether lipid abundances in surface waters and did not detect ether lipids exclusive to the terrestrial lineage, group I.1b Thaumarchaeota (SAGMCG-1 does not produce unique ether lipids), such as methylated 1G- or 2G-GDGTs, unsaturated methoxy archaeols or high abundances of GDGT-4 (>40% relative abundance) and crenarchaeol isomer (>20% relative abundance) as observed for *N. viennensis* soil strains (Elling et al., 2017).

It is noteworthy that previous water column studies in OMZs have reported a significantly lower BHP structural diversity (Berndmeyer et al., 2014; Blumenberg et al., 2007; Kharbush et al., 2013; Sáenz et al., 2011; Wakeham et al., 2007) than observed in this study. We attribute this increase in the number of identified structures to the higher sensitivity and resolving power of the Orbitrap system used for analysis.

To our knowledge, genomic data characterizing the microbial community in Effingham Inlet and Deer Bay have yet to be determined and our sample sizes were too small to obtain genomic data (samples for genomics only were not obtained at the time of sampling). However, hydrochemical and genomic data are available for two neighboring fjords on Vancouver Island, Nitinat Lake, and Saanich Inlet (Figure 1) (Michiels et al., 2019; Pawlowicz et al., 2007; Schmidtova et al., 2009; Torres-Beltrán et al., 2016; Zaikova et al., 2010). Nitinat Lake and Saanich Inlet are also shallow (up to ~200m water depth) stratified fjords that are separated from water exchange with the Pacific through sills, and their anoxic waters are episodically overturned (Pawlowicz et al., 2007; Torres-Beltrán et al., 2017). Given the similarity in oceanographic conditions, both Nitinat Lake and Saanich Inlet are likely close analogs for our settings; thus, in the following discussion, we compare our data with evidence previously obtained from these two locations while acknowledging the likely occurrence of spatial and temporal changes in the microbial community.

5.1 | Ammonium oxidation

5.1.1 | Archaeal ammonia oxidation

High crenarchaeol (cren) abundances within the core GDGT (43.0 ± 1.4%; mean ± SD), 1G-GDGT (45.9 ± 2.9%), and
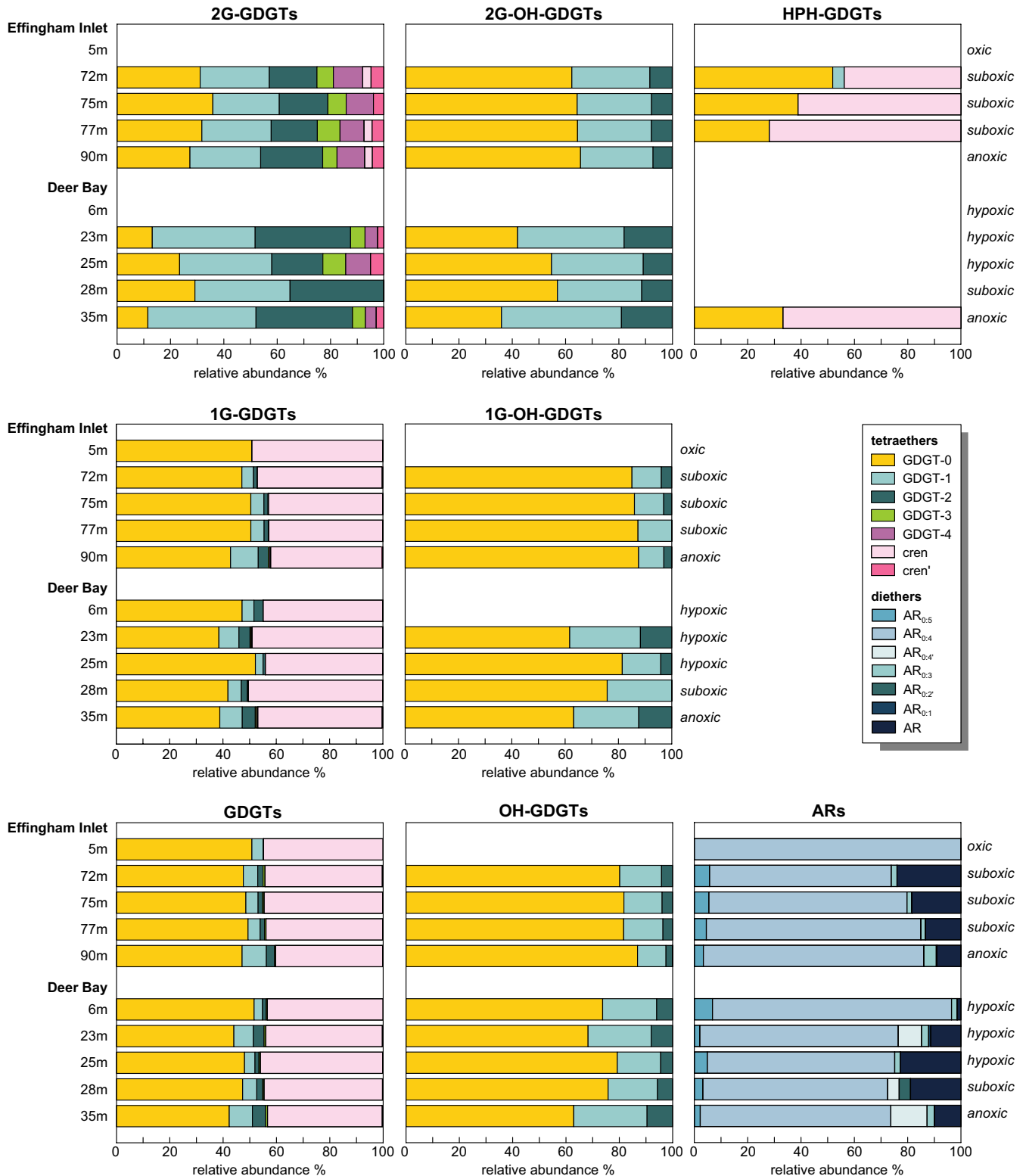
FIGURE 5 Relative abundance of alkyl chain structural types in IPL (2G-GDGTs, 2G-OH-GDGTs, HPH-GDGTs, 1G-GDGTs, and 1G-OH-GDGTs) and CL (GDGTs, OH-GDGTs, and ARs) archaeal lipid classes detected in the Effingham Inlet and Deer Bay water columns

HPH-GDGT (60.9 ± 10.6%) populations suggest that ammonia-oxidizing Thaumarchaeota are likely the dominant source of ether lipids in both Effingham Inlet and Deer Bay (Elling et al., 2017), consistent with observations from Saanich Inlet where they contribute

up to ~28% of the total microbial community and >90% of the archaeal community at various depths (Michiels et al., 2019). A significant ($p < .05$) positive correlation between most tetraethers in Effingham Inlet and Deer Bay (Figure 6) further points to a common
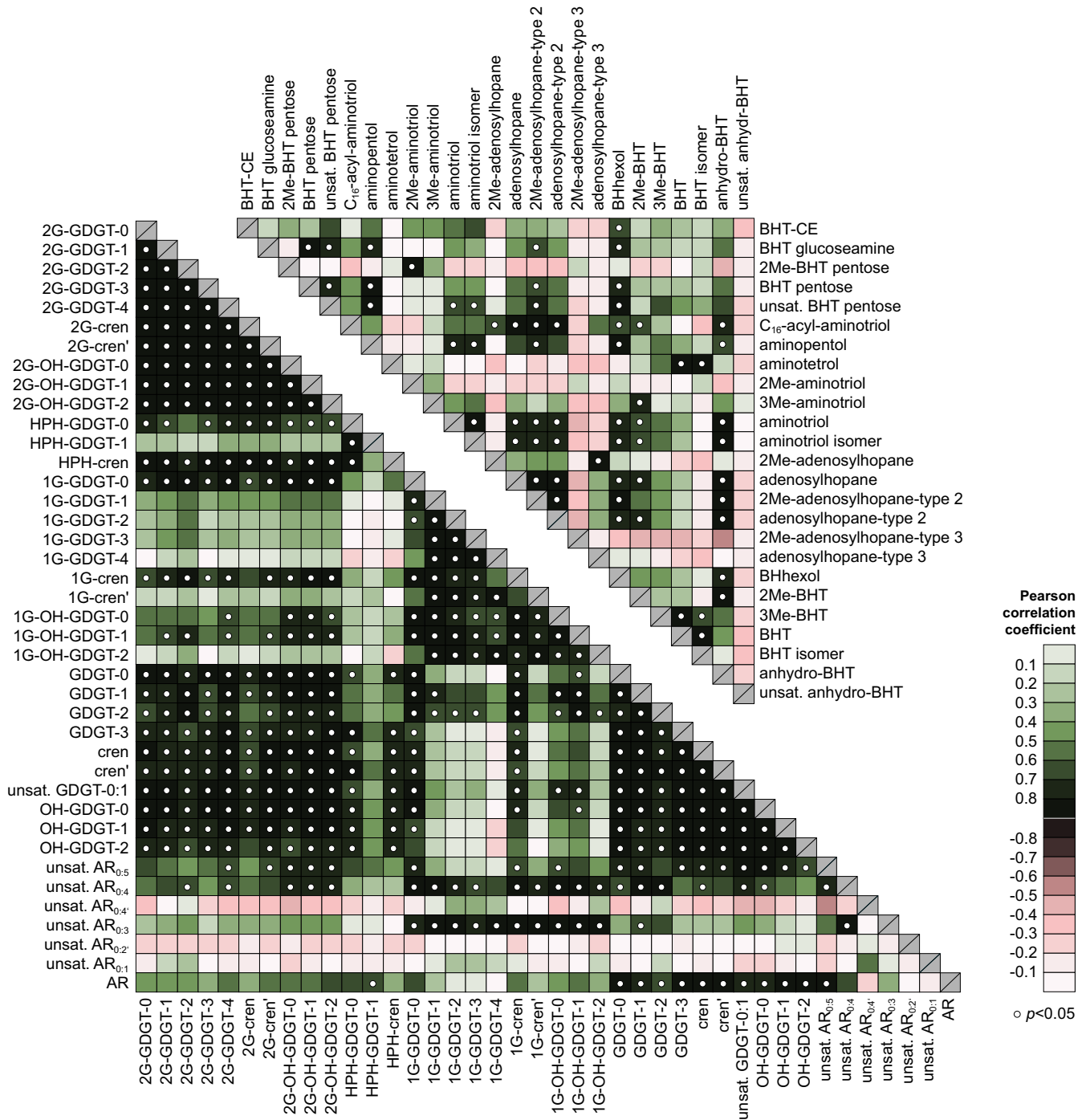
FIGURE 6 Pearson correlation matrix between bacterial or archaeal biomarkers in the Effingham Inlet and Deer Bay water columns

origin from group I.1a Thaumarchaeota. In addition to compounds produced by several thaumarchaeal lineages, we detect ether lipids that among the Thaumarchaeota are exclusively produced by group I.1a, such as 2G-OH-GDGT-0 (diglycosyl-hydroxy-GDGT-0), 2G-OH-GDGT-1, OH-GDGT-0, OH-GDGT-1, and OH-GDGT-2 (Figure 6; Elling et al., 2017), although they have also been identified in *Methanothermococcus thermolithotrophicus* (Liu et al., 2012). A group I.1a Thaumarchaeota origin is also supported by cren'/(cren'+cren) ratios (cren'= crenarchaeol isomer) of <0.01 (according

to Sinninghe Damsté et al., 2012). Comparison with the published lipidome of group I.1a Thaumarchaeota reveals that the IPL diversity at our study sites (Figure 4) most closely resembles that reported for *Nitrosopumilus maritimus* (Elling et al., 2014, 2017), which is characterized by higher 1G-GDGT abundances (up to ca. 50%–60%) than other species. A primary origin from either the *Nitrosopumilus*-like clade or the water column group A (WCA) clade of the group I.1a Thaumarchaeota would be expected from marine ecotype depth distributions in the water column (Beman et al., 2008; Reji, Tolar, Smith,

Chavez, & Francis, 2019a, 2019b; Santoro et al., 2019). However, the relative abundance of 1G-GDGT in *N. maritimus* is not as high as what we found in the Effingham Inlet and Deer Bay water columns (>75%). Furthermore, the poor correlation between 1G-GDGTs and other ether lipids (Figure 6) as well as the high abundances of intact and core non-cyclized GDGT-0 in both inlets likely indicate an additional (though likely minor) contribution of anoxygenic euryarchaeal ether lipids. We discuss this aspect in Section 5.3.1.

The abundance of HPH-crenarchaeol, the most labile and representative IPL of viable Thaumarchaeota (for details see Elling et al., 2014; Schouten et al., 2012), suggests that these organisms primarily reside or are active in suboxic waters in Effingham Inlet and in anoxic waters in Deer Bay, respectively (Figure 2f,l). We of course acknowledge that the boundary between "suboxic" and "anoxic" conditions represents a gradient rather than a sharp contrast. In fact, HPH-crenarchaeol peaks at the lower boundary of the suboxic zone in Effingham Inlet, whereas it could only be detected in anoxic waters in Deer Bay (Figures 2 and 5). The latter sample, however, was obtained from just below the suboxic zone, so it might actually have low O_2 concentrations around or below the ~1 µM detection limit. This pattern is consistent with evidence from the Arabian Sea OMZ, where Pitcher et al., (2011) found highest thaumarchaeal 16S rRNA and 16S rDNA as well as *amoA* gene abundances in suboxic waters, which were tracked by elevated HPH-crenarchaeol abundances. 16S rRNA results from other OMZ settings also show that MG I Thaumarchaeota are abundant in suboxic waters in the Baltic Sea (Labrenz et al., 2010), the Black Sea (Sollai et al., 2019), and the Eastern Tropical South Pacific (ETSP) (Sollai et al., 2019). In particular, *Nitrosopumilus* has been shown to dominate the suboxic Black Sea archaeal community and even to persist into euxinic waters (Sollai, Villanueva, Hopmans, Reichart, et al., 2019). In the Black Sea and ETSP, absolute HPH-crenarchaeol abundances were highest in oxic surface waters, which is most likely explained by the high abundance of *Ca*. Nitrosopelagicus (WCA clade) in addition to *Nitrosopumilus* in those waters. In comparison, Sollai et al., (2015) found the HPH-crenarchaeol peak in suboxic to anoxic waters of the Eastern Tropical North Pacific (ETNP) OMZ. This is in agreement with data from Schubotz et al., (2018) further south in the ETNP, where HPH-GDGTs accumulate in the core OMZ and the deep oxycline. Ammonia-oxidizing archaea (AOA) have also been shown to maintain high activity at <6 µM O_2 in culture (Qin et al., 2017); thus, AOA-derived gene copies or biomarkers detected in suboxic or anoxic waters likely represent viable cells rather than result from export and preservation of HPH-GDGTs from overlying water masses.

Although HPH-GDGTs were not detected in the surface waters of either Effingham Inlet or Deer Bay, diether ARs, particularly $AR_{0:4}$, are prominent (Figures 2e,k and 4). Indeed, $AR_{0:4}$ represents 19% and 24% of all CLs in the respective surface water sample of Effingham Inlet and Deer Bay, and only 2%–5% of tetraether CLs at depth in both inlets. $AR_{0:1}$ and $AR_{0:2}$ are produced by group I.1a Thaumarchaeota (Elling et al., 2017), and Zhu et al., (2016) had previously concluded that $AR_{0:4}$ in epipelagic oxic environments may be a biomarker for photoheterotrophic Marine Group II (MG II) Euryarchaeota. High

abundances of unsaturated ARs have been identified in *Thermoplasma acidophilum*, a cultivated Euryarchaeote closely related to MG II, and HPH-GDGT-0 (as well as HPH-crenarchaeol) correlated with MG II OTU-1 gene reads in the Black Sea water column (Sollai, Villanueva, Hopmans, Reichart, et al., 2019), a pattern that could be expected from photoheterotrophic Euryarchaeota. In Saanich Inlet, Michiels et al., (2019) identified Euryarchaeota in surface waters, where they could represent MG II. However, absolute $AR_{0:4}$ concentrations in both Effingham Inlet and Deer Bay are lowest in surface waters and increase with depth, and they correlate significantly with most ether lipids (Figure 6). The latter suggests that $AR_{0:4}$ might in fact also be produced by MG I. This interpretation is consistent with recent evidence challenging ether lipid production by MG II (Zeng et al., 2019). These authors demonstrated that MG II lack the GrsA and GrsB proteins responsible for the synthesis of cyclized GDGTs. While this fact does not exclude their ability to synthesize acyclic GDGT-0 or ARs, Besseling et al., (2020) showed that SPM from MG II-dominated surface waters in the North Atlantic Ocean and the coastal North Sea did not contain any known tetraether or diether IPLs, nor did they yield notable amounts of GDGTs or ARs following acid hydrolysis. Only those samples characterized by >10% thaumarchaeal 16S rRNA gene abundance also contained archaeal lipids (Besseling et al., 2020). Accordingly, the majority of cyclized ether lipids as well as $AR_{0:4}$ in surface and deeper waters in Effingham Inlet and Deer Bay probably have a common MG I thaumarchaeal source with a preferred suboxic-anoxic niche. Different absolute and relative abundances in surface waters may stem from adaptations to changes in environmental conditions such as temperature, salinity, and light as shown for laboratory cultures (Elling et al., 2014; Qin et al., 2014).

5.1.2 | Bacterial anaerobic ammonium oxidation (anammox)

Michiels et al., (2019) showed that Planctomycetes are present throughout the Saanich Inlet water column and that anammox contributes ~37% of the annual N loss in this area. It is therefore likely that anammox bacteria also play a prominent role in the N cycle of the analogous environments of Effingham Inlet and Deer Bay. BHT-x, the BHT isomer exclusively produced by anammox bacteria (Schwartz Narbonne et al., 2020), can be used to trace this process in our water column samples. While our chromatographic method did not resolve BHT-II and BHT-x (Schwartz Narbonne et al., 2020), it is likely that the low isomer abundances detected above ~30–40 µM O_2 (in the surface water samples) reflect "background" BHT-II produced by other bacteria, and the substantially higher isomer abundances below this threshold could then be attributed to an additional contribution of BHT-x to "background" BHT-II (Figure 7). Kalvelage et al., (2011) report the upper limit for anammox to be ~20 µM O_2, which agrees well with the half-inhibition concentration (IC_{50}) for anammox between 1 and ~10 µM O_2 determined by Bristow et al., (2016). In contrast, Dalsgaard et al. (2014) showed that the anammox IC_{50} in the Chilean OMZ is
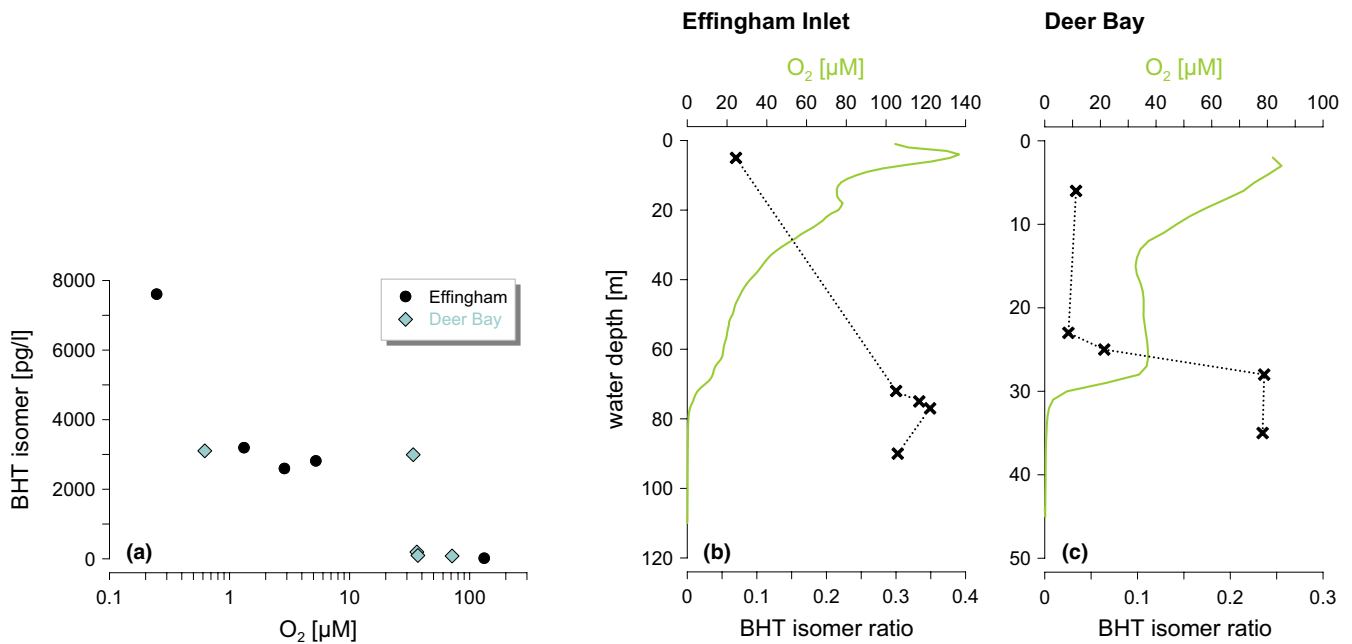
FIGURE 7 BHT isomer distribution in the Effingham Inlet and Deer Bay water column. (a) cross plot of absolute BHT isomer abundance and oxygen concentrations; (b) and (c) BHT isomer ratio (BHT isomer/[BHT + BHT isomer]) in Effingham Inlet and Deer Bay

approximately one order of magnitude lower (886 nM O_2). The observations of Kalvelage et al., (2011) and Bristow et al., (2016) agree well with BHT isomer concentrations along the O_2 concentration gradient in our samples, which are below 100 pg/L above ~30–40 μM O_2, and increase to >2.5 ng/L between 0.3 and 30 μM O_2 and eventually reach up to 7.6 ng/L below ~0.3 μM O_2 (Figure 7a). Accordingly, BHT–x concentrations would be approximately 1–2 orders of magnitude higher than "background" BHT -II concentrations in both inlets, and, thus, BHT isomer abundances (Figures 2 and 3) primarily reflect abundances of anammox bacteria.

Prior to the detection of BHT-x, BHT-II ratios (BHT-II/ [BHT + BHT-II]) were used to detect anammox rather than absolute BHT-II concentrations since BHT-II is also observed in oxic environments (Kusch et al., 2018, 2019; Matys et al., 2017; Sáenz et al., 2011). In Effingham Inlet and Deer Bay, the BHT isomer ratios (BHT isomer/[BHT + BHT isomer]) are significantly higher below the redoxcline (Figure 7b,c) and are within the range reported for water column SPM and core-top sediments in the Arabian Sea by Sáenz et al., (2011). Yet, BHT isomer ratios in Effingham Inlet and Deer Bay are lower (0.07–0.35 and 0.03–0.24, respectively) than the values reported in water column SPM from other marine OMZ settings supporting anammox, such as the Chilean Margin (Matys et al., 2017), the Peru Margin and Cariaco Basin (Sáenz et al., 2011), as well as in sediments underlying the OMZ systems of the Arabian Sea (Lengger et al., 2019) and northern Chile (Matys et al., 2017). In these settings, BHT isomer ratios typically range between 0.4 and 0.6, close to the ratios (0.55–0.64) observed in *Ca*. Scalindua profunda enrichment cultures (Rush et al., 2014). Lower BHT isomer ratios in Effingham Inlet and Deer Bay may stem from additional in situ production of BHT by other types of bacteria, or potentially, as a result of transport from the catchment (although as outlined above, we expect it to be

small). Additional in situ production would explain the lower BHT isomer ratios in Deer Bay, for which an additional source of BHPs is implied by the high aminotriol abundances (Figures 2c,i and 3).

We observe the highest BHT isomer abundances in the anoxic samples in Effingham Inlet Deer Bay, but it is unclear whether these samples were also sulfidic. Unfortunately, HS⁻ was not monitored during CTD deployments in Effingham Inlet and Deer Bay, but the characteristic smell was observed during the cruise and previous hydrochemical data from Effingham Inlet and Nitinat Lake show an increase in HS⁻ concentrations immediately below the suboxic zone or, at times, even at the bottom of the suboxic zone (Hurtgen et al., 1999; Pawlowicz et al., 2007). H_2S concentrations of up to 160 μM have been measured at the sediment-water interface in Effingham Inlet (Hurtgen et al., 1999; Ingall et al., 2005). Anammox is sensitive to HS⁻ (Dalsgaard et al., 2003), but anammox bacteria seem capable to persist under slightly sulfidic conditions (e.g., Hawley et al., 2014; Michiels et al., 2019; Sollai, Villanueva, Hopmans, Reichart, et al., 2019). We note that Planctomycetes 16S rRNA genes and proteins involved in the putative Planctomycete reductive acetyl-CoA pathway have been detected throughout the water column in Saanich Inlet, including in sulfidic bottom waters (Hawley et al., 2014; Michiels et al., 2019), whereas the addition of HS⁻ to Saanich Inlet water samples inhibited anammox at concentrations >2.5 μM (Michiels et al., 2019). Thus, an additional (syntrophic) mechanism such as chemolithoautotrophic denitrification (Russ et al., 2014; Wenk et al., 2012) is required to explain the persistence of anammox at depth in both Effingham Inlet and Deer Bay. Chemolithoautotrophic denitrification by sulfur-oxidizing γ- and ε-proteobacteria can detoxify HS⁻ (Lavik et al., 2009) and in turn provide NH_4^+ or NO_2^- to anammox in what has been described as a "cryptic sulfur cycle" even while other

terminal electron acceptors are present (Canfield et al., 2010). This metabolism has indeed been determined to be the most prominent chemoautotrophic process in OMZ waters off northern Chile based on a stable isotope box model (Vargas et al., 2021). In fact, an isolate of the SUP05 cluster (*Ca*. Thioglobus autotrophica strain EF1) that was recently obtained from the upper suboxic zone in Effingham Inlet has been characterized as an anaerobic sulfur-oxidizing chemolithoautotroph consuming NH_4^+ at a kinetic rate much lower than ammonia oxidizers, thus, potentially providing NO_2^- for anammox (Shah et al., 2016).

5.2 | Bacterial sulfate reduction and/or sulfur oxidation

Increasing evidence suggests that the nitrogen and sulfur cycles are intimately linked in OMZs, and that sulfur-oxidizing bacteria in addition to sulfate-reducing bacteria (SRB) are active in this "cryptic sulfur cycle" across redox boundaries (Canfield et al., 2010; Ulloa et al., 2012). Among the most frequently observed gene sequences in OMZs are those belonging to the γ-proteobacterial SUP05 cluster and the δ-proteobacterial Desulfobacteraceae (Wright et al., 2012). Both sulfur-oxidizing SUP05 and SRB have been identified in the water column of Nitinat Lake on Vancouver Island (Schmidtova et al., 2009), although these authors concluded that sulfate reduction is rather negligible. In Saanich Inlet, subsurface waters harbor δ-proteobacteria but are dominated by γ-proteobacteria that primarily belong to SUP05, and their abundances increase with depth (Hawley et al., 2014; Michiels et al., 2019; Walsh et al., 2009). SUP05 has been proposed to be driving denitrification in Saanich Inlet in a consortium with Marinimicrobia, particularly during stagnant times (Hawley et al., 2014, 2017; Michiels et al., 2019). Thus, it is likely that both sulfur-oxidizing and sulfate-reducing bacteria also play prominent roles at the redox boundaries of Effingham Inlet and Deer Bay.

Sulfate-reducing bacteria of the genus *Desulfovibrio* have been shown to produce the BHPs aminopentol and aminotetrol (Blumenberg et al., 2006, 2009, 2012), often associated with aerobic methane oxidation (Rush et al., 2016 and references therein). Typically, the studied *Desulfovibrio* SRB produce aminopentol and aminotetrol at characteristically low to trace abundances relative to aminotriol, while aminotriol abundances are low in most methanotrophs that produce aminopentol and aminotetrol (Rush et al., 2016). Several studies have, thus, used ratios of aminotetrol:aminotriol and aminopentol:aminotriol to assess potential contributions from *Desulfovibrio* species to aminotetrol and aminopentol pools in environmental samples (Rush et al., 2016; Talbot et al., 2014, 2016). Our Vancouver Island samples provide an opportunity to test this approach in a setting that likely harbors SRB; however, we acknowledge that these ratios should be interpreted with caution. Aminopentol:aminotriol ratios in *D. salexigens* were ~1:1,350 (Blumenberg et al., 2012), whereas aminotetrol:aminotriol ratios ranged between 1:20 and 1:100 in *Desulfovibrio* isolates from a Black Sea microbial mat (Blumenberg et al., 2006), or >1:190 in a *D. bastinii*

isolate (Blumenberg et al., 2009). In contrast, aminopentol:aminotriol and aminotetrol:aminotriol ratios in methanic marine sediments and carbonates, for example, averaged 1:17 and 1:14, respectively, although ratios as low as 1:112 and 1:110, respectively, were observed (Rush et al., 2016). In Deer Bay, aminotriol abundances are particularly high below surface waters (Figures 2c,i and 3), but aminopentol was only detected at 28 and 35 m water depth. Corresponding aminopentol:aminotriol ratios of about 1:5,680 and 1:9,860 at 28 and 35 m depth, respectively, as well as aminotetrol:aminotriol ratios of up to 1:215 at 35 m depth (Table S1) may, thus, indicate the presence of *Desulfovibrio* sp. in the deep Deer Bay. The highest aminotetrol:aminotriol ratios in Deer Bay (1:44 at 6 m depth and 1:77 at 23 m depth) fall within the range observed in methanic settings by Rush et al., (2016), suggesting multi-modal sources including methanotrophs and SRB. However, it should be noted that *Desulfovibrio* sp., classified as obligate anaerobes, have frequently been observed in oxygenated marine settings including fjords (e.g., Teske et al., 1996) and shown to grow under nearly atmospheric O_2 conditions (Lobo et al., 2007). In samples where aminopentol and aminotetrol were detected, both ratios show an overall decrease with depth and high absolute abundances of aminopentol and aminotetrol in suboxic/anoxic waters support the argument that both amino-BHPs at these depths are not derived from aerobic methanotrophs but are rather linked to anoxic metabolisms such as sulfate reduction. If the high aminotriol abundances primarily reflect SRB production in Deer Bay, this result is in stark contrast to nearby Nitinat Lake where dissimilatory sulfite reductase (*dsr*) gene abundances and $^{35}SO_4^{2-}$ tracer experiments revealed the near-absence of SRB (Schmidtova et al., 2009), and to Saanich Inlet where SRB-defined proteins could not be detected in the metaproteome (Hawley et al., 2014). Instead, sequences from a novel ε-proteobacterial *Arcobacter* sp. group (NITEP5) together with *Sulfurimonas* sp. and SUP05 sequences were identified to be dominant in anoxic waters of Nitinat Lake, implying the presence of active sulfur-oxidizing bacteria. *Arcobacter* is also important in Saanich Inlet although its occurrence has been linked to NO_3^- input during water renewal events (Michiels et al., 2019). Due to the absence of cultured representatives of the SUP05 cluster (except *Ca*. Thioglobus autotrophica strain EF1 (Shah et al., 2016)) and the Nitinat NITEP5 *Arcobacter* group, these bacteria have not been studied for BHP production. Nonetheless, proteobacteria are among the most prolific BHP producers (Kannenberg & Poralla, 1999). However, at least SUP05 is present in Effingham Inlet (Shah et al., 2016), where aminopentol was absent and aminotetrol:aminotriol ratios are high (1:6 to 1:8), suggesting that SUP05 likely does not produce unusual BHP signatures. High aminotetrol:aminotriol ratios also suggest that aminotetrol does not derive from *Desulfovibrio* sp. in this setting. Since aminotetrol shows the same depth pattern as aminotriol in Effingham Inlet (Table S1), it seems likely that both BHPs are produced by methanotrophs (upper water column) or other (non-specific) bacteria, and that their increase with depth mirrors the overall increase in bacterial abundance and dark carbon fixation. Our BHP results, thus, indicate that *Desulfovibrio* SRB may be present in the anoxic deep waters of Deer Bay. On the other hand, amino-BHP ratios suggest

their absence from the anoxic waters of Effingham Inlet at the time of our sampling. Nonetheless, additional data on BHP production by other SRB species/genera are required to fully assess the feasibility of using amino-BHP ratios to trace sulfate reduction.

5.3 | Methanogenesis and methane oxidation

5.3.1 | Archaeal methanogenesis and/or methanotrophy

Stratified water bodies that are characterized by diverse anaerobic metabolisms are also important environments within the global methane cycle (Wright et al., 2012). Anoxic and sulfidic waters accumulate high levels of methane from methanogenesis in underlying sediments, although sinking particulate matter has also been shown to provide micro-niches for methanogens in the water column (Karl & Tilbrook, 1994). Anaerobic oxidation of methane is prevalent in anoxic waters, including coastal fjords (Ward & Kilpatrick, 1990; Ward et al., 1989), and is a likely mechanism that dampens efflux of methane—an important greenhouse gas—to the atmosphere (Reeburgh, 2007; Ward et al., 1989). Both methanogenic and methanotrophic Euryarchaeota inhabit oxygen-depleted waters and their ether lipid compositions differ from that of pelagic Thaumarchaeota, as well as from one another (Blumenberg et al., 2004; Koga et al., 1993, 1998; Kurth et al., 2019; Rossel et al., 2011; Schouten et al., 2013).

As mentioned above (Section 5.1.1), although the ether lipid distribution in Effingham Inlet and Deer Bay is similar to that of *N. maritimus* (Elling et al., 2014; Schouten et al., 2008), we do observe some distinct differences. Compared to group I.1a Thaumarchaeota (Elling et al., 2017), the distribution of tetraethers in both inlets is characterized by overall higher abundance of 1G-GDGTs and a dominance of GDGT-0 within the IPL and CL pools, respectively (Figures 4 and 5). Among CLs, GDGT-0 represents 47.7 ± 2.7% (mean ± SD) of CLs, whereas GDGT-1, GDGT-2, and GDGT-3 contribute only 8.4 ± 3.2% of CLs in both inlets (Figure 5). OH-GDGTs (76.7 ± 7.0%), 1G-OH-GDGTs (78.5 ± 9.9%), and 2G-OH-GDGTs (55.8 ± 10.5%) are also dominated by non-cyclized GDGT-0, and 2G-GDGT-0 contributes substantially (up to 35.9%) to 2G-GDGTs (Figure 5) in Effingham Inlet and Deer Bay, whereas it is virtually absent from *N. maritimus* (Elling et al., 2014). Archaeol and GDGT-0 occur in high abundance in methanogenic Euryarchaeota (Koga et al., 1993, 1998), and hydroxylated GDGTs have been detected in *Methanothermococcus thermolithotrophicus* (Liu et al., 2012). In Effingham Inlet and Deer Bay, OH-GDGT-0 contributes up to 6.8% and 7.4% of total ether lipids, respectively (Table S2), and other OH-GDGTs only occur with up to two cyclopentane moieties (Figure 5), similar to what has been reported in Euryarchaeota (Liu et al., 2012). These lines of evidence suggest that methanogenic Euryarchaeota contribute to the ether lipid pool in our samples. However, archaeol abundances in Effingham Inlet and Deer Bay are mostly below 1% of total ether lipids (Figure 4,5), similar to observations for group I.1a Thaumarchaeota (Elling et al., 2017);

hydroxyarchaeol was not detected in Effingham Inlet and Deer Bay although it is common in methanogens (Koga et al., 1993, 1998). GDGT-0/crenarchaeol ratios in both Effingham Inlet and Deer Bay range from 1.0 to 1.2, that is, are lower than the suggested threshold value (≥2.0) indicating a methanogenic origin (Blaga et al., 2008).

GDGT-0 and archaeol are, however, also common in anaerobic methane-oxidizing archaea (ANME), which primarily occur in consortia with SRB (Boetius et al., 2000; Hinrichs et al., 1999). ANME ether lipids seem distinct among clades (Blumenberg et al., 2004; Kurth et al., 2019; Rossel et al., 2011). For instance, Rossel et al., (2011) showed that ANME-1 synthesize substantial amounts of 2G-GDGTs, whereas ANME-2 and ANME-3 produce glycosidic and phosphatidic diethers. Sollai, Villanueva, Hopmans, Reichart, et al., (2019) reported a strong correlation of diether IPLs with ANME-1b in the Black Sea water column. In Effingham Inlet and Deer Bay, 1G-GDGTs rather than 2G-GDGTs occur in much higher relative abundance than in group I.1a Thaumarchaeota (Elling et al., 2017); only core diethers were detected, and archaeol abundances were low. Moreover, GDGT-1 and GDGT-2 are not notably high, although they typically are abundant in anaerobic methane-oxidizing archaea (Zhang et al., 2011) and have been attributed an ANME-1 origin in the anoxic Black Sea water column (Wakeham et al., 2007). Lastly, GDGT-2/cren ratios below 0.12 do not indicate a methanotrophic origin (GDGT-2/cren ≥0.2; Weijers et al., 2011) for the ether lipids in Effingham Inlet and Deer Bay.

Based on these several lines of evidence, overall contributions from methanogenic or methanotrophic Euryarchaeota to the ether lipid pool seem to be rather low. This observation is consistent with results for Saanich Inlet, where ammonia oxidation rates are higher than methane oxidation rates (Ward & Kilpatrick, 1990), and where thaumarchaeal 16S rRNA gene copies predominate over euryarchaeal 16S rRNA gene copies (Michiels et al., 2019; Torres-Beltrán et al., 2016; Zaikova et al., 2010).

5.3.2 | Bacterial methanotrophy

Aminopentol and aminotetrol are often used as biomarkers for Type I and Type II aerobic methanotrophs, respectively (Spencer-Jones et al., 2017; Talbot et al., 2001; van Winden et al., 2012), although not all Type I and Type II methanotrophs synthesize aminopentol or aminotetrol, respectively (Rush et al., 2016). The absence of aminopentol does not, however, a priori imply the absence of bacterial methanotrophy. Other BHPs have also been linked to methanotrophic bacteria, including methylcarbamate-substituted amino-BHPs (Rush et al., 2016) or BHPs with C-3 methylation such as 3Me-aminotriol and 3Me-BHT (Neunlist & Rohmer, 1985; Rush et al., 2016; Zundel & Rohmer, 1985). However, the latter are not exclusively produced by methanotrophs. Moreover, Welander and Summons (2012) have shown that taxonomically diverse groups contain the hopanoid C-3 methylase (*hpnR*) including many that are not methanotrophic.

In Effingham Inlet and Deer Bay, we expect that potential BHP production by in situ methanotrophic bacteria would primarily occur in

suboxic waters, analogous to Saanich Inlet (Torres-Beltrán et al., 2016; Ward & Kilpatrick, 1990; Ward et al., 1989). In Saanich Inlet, Type I methanotrophic Methylococcaceae and *Methylomonas*, potential sources of aminopentol, were identified in the microbial community (as well as methanotrophs belonging to OPU1 and OPU3, and mussel symbionts), although at <1% relative abundance (Torres-Beltrán et al., 2016; Zaikova et al., 2010). Any supply of wetland-derived methanotroph BHPs (rather than in situ produced) should be mirrored by accumulation in surface waters (due to water column stratification and remineralization), although wetland coverage (peat bogs, marshes, and swamps) around the fjords is sporadic (British Columbia Wetlands Atlas; https://cmnmaps.ca/WETLANDS/). However, aminopentol is absent from Effingham Inlet, whereas aminotetrol, which is not detected in oxic surface waters, instead shows highest concentrations in the anoxic sample (Table S1). In Deer Bay, aminopentol and aminotetrol show highest abundances just above or in the upper suboxic zone; on the other hand, high aminotriol abundances that correlate significantly with aminopentol (Figure 6) and low aminotriol:aminopentol ratios suggest that both BHPs may in part also derive from *Desulfovibrio* SRB, in addition to methanotrophs, as discussed above (Section 5.2), particularly in the deepest samples. Methylcarbamate-substituted amino-BHPs were not detected in either of the fjords, and the highest abundance of 3Me-BHT that occurs in anoxic samples correlates positively with BHT and BHT isomer (Figure 6), that is, may have a non-specific origin. 3Me-aminotriol in the Effingham Inlet water column shows a depth pattern that differs from aminotetrol, aminotriol, and 3Me-BHT, that is, 3Me-aminotriol shows highest abundances at the bottom of the suboxic zone similar to 2Me-aminotriol and BHhexol (Table S1). BHhexol has thus far only been detected in the anaerobic methanotroph *Methylomirabilis oxyfera* (Kool et al., 2014) and the thermophilic *Alicyclobacillus acidoterrestris* (Řezanka et al., 2011). In Saanich Inlet, however, particulate methane monooxygenase (*pmoA*) gene phylogeny did not reveal any relationship between the observed methanotrophic sequences and *Methylomirabilis oxyfera* (Torres-Beltrán et al., 2016). In our samples, BHhexol correlates significantly with various other BHPs (Figure 6). In addition to BHhexol in the Effingham Inlet water column, several composite BHPs, including BHT-CE, 2Me-BHT pentose, BHT pentose, and unsaturated BHT pentose, show depth distributions similar to 3Me-aminotriol (Table S1). However, none of these BHPs are taxonomically specific and may derive from other, yet uncharacterized bacterial sources. Accordingly, assuming that methanotrophic bacteria are present in Effingham Inlet and Deer Bay, BHP contributions from these bacteria are likely too low to notably imprint BHP distributions analogous to potential methanotrophic ether lipid contributions, even though ether lipid distributions do indeed point to minor methanogenic contributions.

5.4 | Evaluating potential links between bacterial and archaeal lipid producers

Few studies have linked BHP and ether lipid (particularly IPL) distributions in water column samples (Wakeham et al., 2007, 2012),

and heretofore not at the same level of compound diversity as in the present investigation. The high BHP and ether lipid structural diversity detected in this study allows us to investigate whether specific BHP and ether lipid abundances are correlated in Effingham Inlet and Deer Bay (Figure 8). Correlation of specific BHP and ether lipids, if taken as indicative that the respective microbial sources occupy the same ecological niche, may aid at constraining the sources of some more common and/or orphan ether lipids and BHPs. A conceptual schematic of the biomarker-inferred microbial community structure and water column chemistry in Effingham Inlet and Deer Bay is shown in Figure 9.

In general, ether lipid and BHP distributions in Effingham Inlet and Deer Bay seem to display contrasting patterns, which is most obvious just above the suboxic zone (25 m water depth) in Deer Bay (Figure 2). In both cases, total ether lipid concentrations exhibit an overall increase across these hydrogeochemical zones as BHP concentrations decrease. However, this pattern is neither consistent for all individual BHPs nor between the two investigated fjords, which in part likely results from the different sampling resolution in both fjords. In Effingham Inlet, composite BHPs show highest abundances at 77 m water depth, whereas in Deer Bay composite BHPs decrease in concert with other BHPs. Pearson correlation analysis for both sites (Figure 8) reveals that few, yet prominent patterns exist such that specific ether lipids and BHPs can be grouped in three clusters as explained below.

The first cluster includes 2G-GDGTs, 2G-OH-GDGTs, and HPH-crenarchaeol, which correlate significantly ($p < .05$) with 2Me-BHT pentose and 2Me-aminotriol. As stated above, among the Thaumarchaeota 2G-OH-GDGT-0 and 2G-OH-GDGT-1 are produced exclusively by group I.1a (Elling et al., 2017) and, thus, most likely represent viable AOA. Therefore, we can speculate that the bacterial producers of 2Me-BHT pentose and 2Me-aminotriol share a common ecological niche with AOA. If the observed correlations were simply due to high abundance of heterotrophic bacteria utilizing thaumarchaeal biomass, we would expect to also observe a significant correlation with BHT, the most common BHP. Both BHPs could be synthesized by NO_2^- oxidizing bacteria or chemoautotrophic denitrifiers that utilize the NO_2^- produced by AOA as proposed for Saanich Inlet (Hawley et al., 2014, 2017). Support for a link between the bacterial sources of 2Me-BHT pentose and 2Me-aminotriol and AOA comes from the recent study of Elling et al., (2020), who showed that 2Me-BHP production in *Nitrobacter vulgaris* depends on cobalamin supply from AOA.

The second cluster includes 1G-GDGTs, 1G-OH-GDGTs, GDGT-1, and GDGT-2, which correlate significantly with aminotetrol, 3Me-BHT, BHT, and BHT isomer. As noted above, 1G-GDGT abundances are much higher in the Effingham Inlet and Deer Bay water column than observed in group I.1a Thaumarchaeota or *N. maritimus*, respectively (Elling et al., 2014, 2017), suggesting a multi-modal origin. Their relative abundances are, in fact, higher than observed in any cultured thaumarchaeal lineage other than the terrestrial thermophiles, but this could also be related to a response to environmental parameters and growth stage (Elling

FIGURE 8 Pearson correlation matrix for bacterial and archaeal biomarkers in the Effingham Inlet and Deer Bay water columns
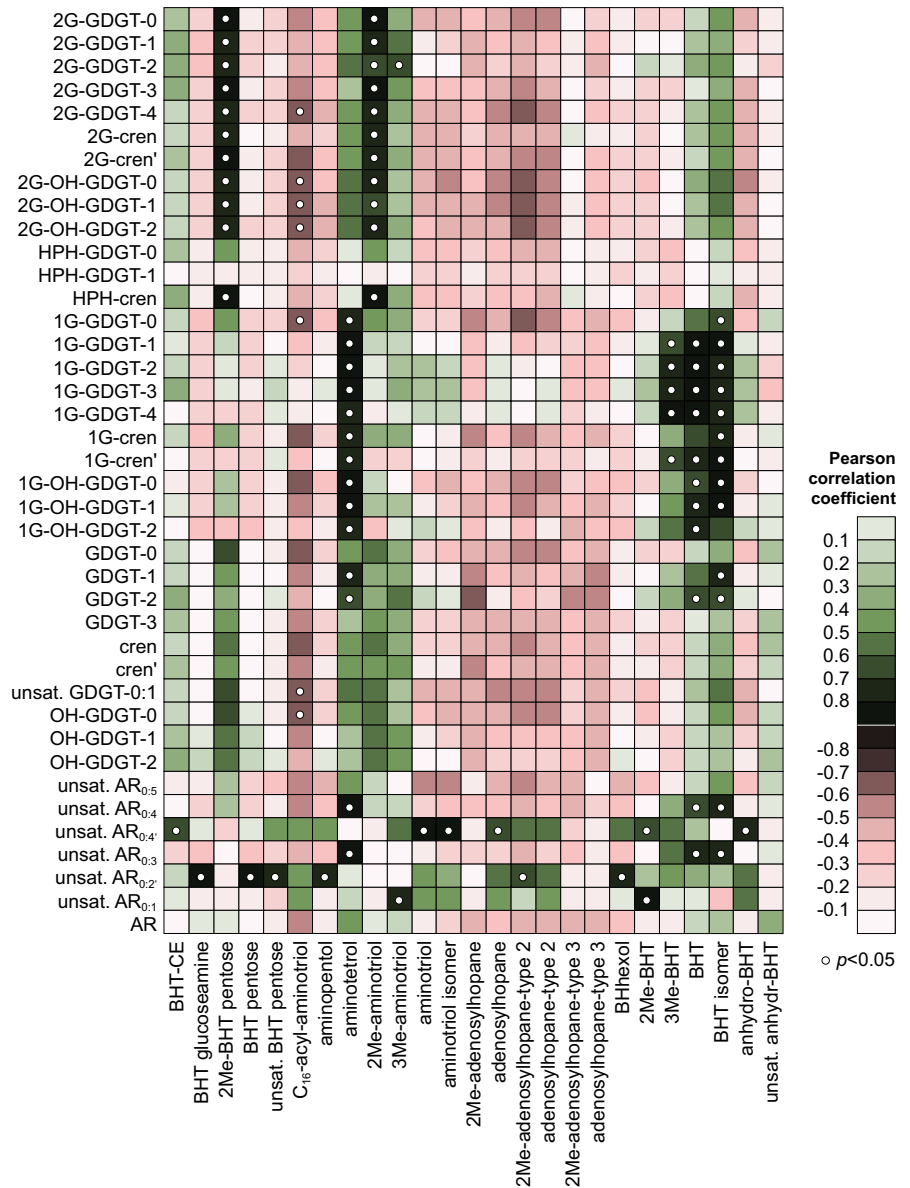
FIGURE 9 Conceptual schematic of the microbial community structure and water column chemistry as inferred by the BHP and ether lipid distribution in Effingham Inlet and Deer Bay at the time of sampling. Boxes with dashed contour lines and italicized font represent organisms for which direct BHP or ether lipid evidence is limited, that is, lipids only indicate minor contributions or source organisms remain to be further constrained
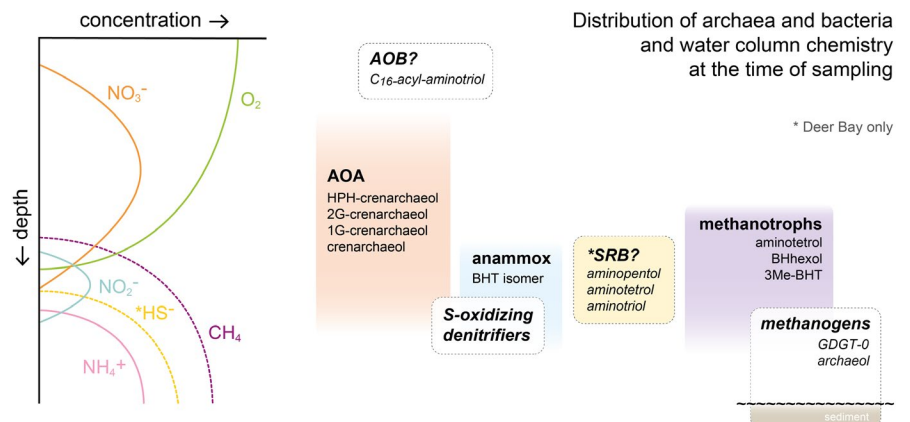
et al., 2014, 2017). A correlation between GDGT-1, GDGT-2, 3Me-BHT, aminotetrol, and BHT isomer suggests that the source organisms share a common niche with anammox bacteria. Although

BHT may primarily be produced by anammox bacteria, we assume that its significant correlation in this cluster also in part mirrors the abundance of heterotrophic bacteria. 1G-GDGTs, 1G-OH-GDGTs,

GDGT-1, GDGT-2, aminotetrol, 3Me-BHT, BHT, and BHT isomer also correlate significantly with $AR_{0:4}$ and $AR_{0:3}$ (Figures 6 and 8). A specific archaeal source organism for the latter, however, remains still elusive (Besseling et al., 2020; Zhu et al., 2016). Our observations suggest that these Archaea thrive under anoxic conditions in Effingham Inlet and Deer Bay and probably do not have an oxygenic photoheterotrophic metabolism.

The third cluster includes 2G-OH-GDGTs, 1G-GDGT-0, 2G-GDGT-4, unsaturated GDGT-0:1, and OH-GDGT-0, which anticorrelate significantly with C_{16}-acyl-aminotriol, suggesting niche partitioning and possibly competition between Thaumarchaea and the C_{16}-acyl-aminotriol source organism(s), which is most abundant in surface waters and likely linked to an oxic metabolism in the water column, or alternatively, supplied from land. Thus far, the only confirmed sources of acyl-aminotriols include purple non-sulfur bacteria (PNSB) and the aerobic ammonium-oxidizing bacterium (AOB) *Nitrosomonas europaea* (Seemann et al., 1999; Talbot et al., 2007). We exclude a PNSB origin based on the oxic conditions in Effingham Inlet and rather consider competition between AOA and AOB as a possible explanation for the observed anticorrelation.

Our correlation analysis also potentially aids at elucidating the niche and sources of the in situ-produced adenosyl-BHPs (Kusch et al., 2021). Adenosylhopane, adenosylhopane-type 2, and 2Me-adenosylhopane-type 2, which Kusch et al., (2021) identified to have a common ecological niche in OMZ settings, correlate significantly with 2Me-BHT, BHhexol, aminotriol and its isomer, and anhydro-BHT in both Effingham Inlet and Deer Bay. Most of these BHPs are rather ubiquitous in bacteria, except BHhexol that has only been identified in methanotrophic *Methylomirabilis oxyfera* (Kool et al., 2014) and thermophilic *Alicyclobacillus acidoterrestris* (Řezanka et al., 2011). A potential methanotrophic origin could be explored in future studies. We note that adenosyl-BHPs show almost no significant correlation with any ether lipids, except adenosylhopane with $AR_{0:4'}$ and 2Me-adenosylhopane-type 2 with $AR_{0:2'}$, but both AR producers are yet unknown. Most adenosyl-BHPs rather show anticorrelation (although not significant) with ether lipids, suggesting either distinct niche separation between the respective producers or, alternatively, strong competition between the respective bacterial and archaeal source organisms.

Whether the observed correlations between BHPs and ether lipids in the three clusters provide means to decipher the sources of BHPs such as 2Me-BHT pentose, 2Me-aminotriol, C_{16}-acyl-aminotriol, and the adenosyl-BHPs should be evaluated by comparison with other stratified water column settings in future studies, ideally in combination with genomic data and high-resolution characterization of the water column chemistry.

6 | CONCLUSIONS

We investigated bacterial BHP and archaeal ether lipid distributions along the stratified water columns of two Vancouver Island fjords, Effingham Inlet and Deer Bay (Tofino Inlet). Our data show that (in the absence of genomic data) the major redox-driven chemo(auto)trophic metabolisms typical for OMZ settings can be traced using these biomarkers (Figure 9). BHPs and ether lipids indicate that the water columns in both fjords are dominated by archaeal and bacterial ammonium oxidizers (group I.1a Thaumarchaeota and anammox bacteria) which are most abundant within or below the redoxcline. These microbes seem to persist under sulfidic conditions as implied by HPH-crenarchaeol and BHT isomer concentrations, suggesting the presence of HS^--detoxifying denitrifiers. In case of the Deer Bay water column, limited BHP evidence might suggest the presence of *Desulfovibrio* (or potentially similar) SRB. Both BHPs and ether lipids also indicate the presence of methanogens (Euryarchaeota) and methanotrophs (possibly including ANME), although these organisms seem to be minor contributors to the overall BHP and ether pools in both fjords.

The unparalleled high BHP and ether lipid structural diversity observed in this study furthermore allowed us to evaluate potential niche overlap or partitioning between the BHP- and ether lipid-producing organisms. We find distinct clusters of significantly correlated individual BHPs and ether lipids that may represent biomarker fingerprints of distinct ecological niches. These correlations should be confirmed by comparison to the ether lipid/BHP signatures in similar environments in future studies. The observed (anti)correlations should also aid in the future identification of (the biogeochemical niche of) the yet unknown source organisms of certain BHPs and ether lipids such as in situ-produced adenosyl-BHPs or unsaturated archaeols.

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SUPPORTING INFORMATION

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