I have reviewed the revised manuscript. It has improved but there remain a number of important issues that still need further attention before this manuscript can be published.

The title of the manuscript still emphasizes the "biogeochemical implications" which are actually fairly minor (see their abstract). The title also claims that it only studies lipids in the OMZ but that is not true. The term "diversity" for lipids seems odd, it is a biological term that should not be used in terms of chemical composition.
We changed the title to "Intact polar lipids in the water column of the Eastern Tropical North Pacific: Abundance and structural variety of non-phosphorus lipids".

The authors have responded in an extensive way to my comments on the analytical methodology. It has become much more clear how the time-line of the analyses has been. They state that they have >10 years of experience with analysis of IPLs and I am not doubting their analytical capabilities at all. However, in the cases where they use rather unusual procedures (Soxhlet extraction of IPLs, re-analysis of IPL extracts after 7 years of storage at -20 degrees centigrade) they have to come up with evidence to back up their statements in the rebuttal and in the text (e.g. lines 195-197; "we believe"). Just the presence of labile IPLs does not mean that the distribution (i.e. relative percentage) has not changed (and it is the distribution that is the focus of the paper as explicitly mentioned in the rebuttal). The authors refer to Lengger et al. (2012) to support this statement. However, this paper uses only Bligh-Dyer extraction. Perhaps, they intended to refer to Lengger et al., Organic Geochemistry 47, 34 – 40 also published in 2012 but this paper shows that the extraction technique has a huge influence on the ratio between glyco- and phospholipids (see Figure 3 in that paper): Bligh Dyer extraction increases the relative amount of the phospholipid by a factor of ten or so. I therefore remain doubtful about the chosen methodology which in view of this is inappropriate.

We meant to cite Lengger et al., 2012 OGC, but mistakenly cited Lengger et al., 2012 GCA, we apologize for this mix-up. We agree with the reviewer that Bligh and Dyer extraction would be the choice method for IPL analysis, however, it is often the case that samples are re-analyzed after newer protocols have been developed and it is not possible anymore for us to perform a different extraction protocol for these samples.
We mention potential extraction issues due to the performed Soxhlet extraction in the revised methods section of the manuscript (lines 196-199) and hope that these now sufficiently address the reviewers concerns.

With respect to the storage issue: why don't the authors show a supplementary figure showing the composition of their IPL standard mixture over time? This would at least solve this issue convincingly with scientific data and would answer my questions is a reasonable way. With respect to the response factors of the standards: I do understand that these response factors are not useful for the community since they are specific for the instrument at that specific moment in time. However, it still would be very helpful to see the change in response factors over time for each individual standard. If the standards are so stable upon storage: why are they renewed every 3 years (which is only 40% of the time between extraction and analysis)?

We added a supplementary figure showing the fluctuations of relative and absolute responses of our different standard mixes over time (Suppl. Fig. 1). The response of each IPL varies over time as the machine is being tuned and cleaned, but there is no obvious indication for preferential degradation of any or all compounds, as absolute and relative responses go up and down (and up again) over the years.

We renew standards not primarily due to concerns of degradation, but mainly because they are either used up or new standards become commercially available. This is also why there is no fixed date when standards get renewed, but always ca. every 2 to 3 years.

A second major point that is not solved in this new version is the distinction between major and minor IPLs (see Figure 4). I reiterate my previous comment: "The whole distinction between major and minor IPLs is rather artificial. It becomes especially confusing when minor IPLs are normalized on

their sum which is a variable part of the IPLs as a whole. It is entirely unclear why this is done other than for "stamp collection" purposes. ".

As we stated in the first response to the reviewer, we visualize the minor lipids, because also minor lipids can be environmentally relevant (cf. laderrane lipids). If we would not zoom into the minor lipids in figure 4 as we do, then changes with depth and zonation would not be visible.

Nevertheless, we agree with the reviewer that scaling the minor lipids up to their sum is arbitrary and may be misleading. Therefore, we now decided to scale the minor lipids to their actual relative abundance to the total IPLs. See revised figure 4.

The text has now been changed to define major and minor IPLs (Lines 309-310) but this definition is rather confusing with the figure because it shows many IPL classes that are <10%. The bar plots of the minor compounds shows really weird things; sometimes there is only one IPL, sometimes none, and the most important thing is that it is normalized on something that is extremely variable (i.e. the sum of "minor" IPLs that varies between 0-20%). So, it is entirely confusing way of plotting the data. If the authors want to focus on these minor IPLs, why don't they use the scale of the plots shown on the left of this figure (IPL relative abundance normalized on the sum of all IPLs) but use a scale that runs from 0-2% or so. That would be a much more fair way to present the data and would also eliminate some of the weird things (i.e. "minor" IPLs dominated by one IPL because in these cases the "minor" IPLs represent <1% of the total).

As the reviewer suggested, we now plotted the minor lipids that they amount to their actual relative abundance compared to total IPLs, see revised figure 4.

This issue directly relates to the problem that the authors keep on insisting in their idea that relative abundances are more important than absolute abundances. The problem they generate in that way is directly evident form their Figure 4. At station 8 archaeal IPLs represent 50-80% of the IPLs whereas this is much lower in the shallower waters. Fig. 2e shows that in the shallower waters there are a comparable or perhaps even higher number of archaeal cells. However, in the shallow waters the IPL concentration is much higher. When absolute concentrations of archaeal IPLs would be taken into consideration this discrepancy would likely not exist. Therefore, I strongly urge the authors to look at their data also in terms of absolute concentrations. Can't they combine their measured absolute total concentrations of IPLs in 2010/2011 (which they now hardly use) with the %IPL determined in 2015 to arrive at concentration profiles for individual IPLs? That would allow a much more meaningful ecological interpretation of their extensive dataset. Of course, percentage data also provides some insight but the basic conclusion from Fig. 4 is now: the photic zone contains predominantly IPLs derived from phytoplankton whereas the IPLs in the OMZ are primarily derived from bacteria and archaea. This is hardly surprising; do we really need all these analyses to arrive at this conclusion?

As stated in the manuscript, the 2010/2011 analyses did not consider HPH-GDGT; therefore, we refrain from reporting total GDGT data from the Xie et al., 2012 paper as these only consider 1G-GDGT and 2G-GDGT.
Unfortunately, the reviewer still seems to misunderstand the main point of the paper if he/she believes it is simply to show that phytoplankton dominate the surface waters while bacteria and archaea dominate the deeper layers. Instead, the main point of the paper is to report on the types of lipids that are found with the different geochemical and biological zones. To us it was surprising that in deeper zones (within the OMZ and below) we find IPLs that were previously not assumed to be typical for bacteria.
Again, we can only re-iterate what we mentioned previously: We do not agree that showing absolute concentrations would gain any more insight into our main point. Furthermore, there are several papers that have reported on absolute concentrations of archaeal lipids in oxygen minimum zones (Pitcher et al., 2011, Schouten et al., 2012), and we thus do not see how our reporting of this would be novel.

I also still believe that correlating environmental parameters to relative abundances of IPLs is much less useful than to absolute concentrations. In any case, the statistical data treatment is not really used

in the discussion, so this section (Lines 406-429) can be easily eliminated.

We would like to keep the statistical evaluation of the data in the manuscript, otherwise we could not make statements about correlations of lipids with environmental parameters, which is surely of interest to many people in this field. Also, it is not true that we do not use the statistical data in the discussion, we do so in many instances in section 4.2.

The discussion has been shortened substantially but is still lengthy. It starts with a paragraph (lines 432-459) that only reiterates previous findings in this setting. Where these previous finding can be directly related to the IPL profiles they should be mentioned there and this paragraph can be skipped.

As this background information reports on previous findings we do believe that the discussion section is the appropriate placing of this relevant information.

There is also overlap in Lines 468-474. I am also puzzled by the fact that in section 4.1.3 IPLs from two completely different zones (core OMZ and deep oxycline, i.e. without and with oxygen) are discussed together.

We inserted a sentence in line 578 explaining why we discuss these two zones together because similar IPL distributions indicate similar biogeochemistries, even though oxygen is rising in the deep oxycline.

The discussion of the origin of the archaeal IPLs (Lines 558-570) is fairly limited. In the upper OMZ hardly any archaeal IPLs are detected (Figure 4) so this needs explanation.

As we report in line 458 (part of a paragraph the reviewer wanted us to delete) Podlaska et al., 2012 showed low archaeal abundances shallower than the upper OMZ, which matches our IPL data.

Also, the reason for linking them to Group II Euryarchaeota needs argumentation.

We revised the sentence where we linked the IPLs to Group II Euryarchaeota (line 572).

The conclusion (line 708-711) is simply wrong. Not only oxygen is the primary determinant but also light. Without light there would not be any production of IPLs by algae and cyanobacteria, quantitatively the most important IPLs in the water column of the studied area.

We now clarify in the text that not only oxygen, but also light drive IPL distribution (lines 265, 477, 657 and 715).

In summary, I strongly feel that this manuscript still needs quite some revision.

We again revised the methods section as well as the discussion and figure 4 according to the reviewers concerns. In addition, we added a supplemental figure showing the fluctuations of the IPL standard response factors over time.

Line 1: The title of the manuscript was changed

Line 33, 35, 143: ‘oxygen minimum zone’ was replaced with ‘water column’ to highlight that we are not only focusing on the OMZ.

Line 203: The methods section was revised to address the Soxhlet extraction issue.

Line 597: Sentence was rephrased according to the reviewer’s request.

Line 603: Sentence was added according to the reviewer’s request.

Lines 277, 687, 745 and 746: Next to high oxygen content also the presence of light in the surface waters is stressed here, according to the reviewer’s request.

We modified Figure 4 and added Suppl. Figure 1 according to the reviewer’s request.

Intact polar lipids in the water column of the Eastern Tropical North Pacific: Abundance and structural variety of non-phosphorus lipids

Florence Schubotz [1*], Sitan Xie [1,¶], Julius S. Lipp [1], Kai-Uwe Hinrichs [1], Stuart G. Wakeham[2]

[1]MARUM and Department of Geosciences, University of Bremen, 28359 Bremen, Germany

[2]Skidaway Institute of Oceanography, Savannah, GA 31411, USA

[¶]Current address: Wai Gao Qiao Free Trade Zone, 200131 Shanghai, China

[*]Corresponding author. MARUM, University of Bremen, Leobener Str. 13, Room 1070, 28359 Bremen, Germany. Tel: +49-421-218-65724. Fax: +49-421-218-65715. E-mail: schubotz@uni-bremen.de

Keywords: intact polar lipids, phospholipids, glycolipids, betaine lipids, ether lipids, oxylipins, phospholipid substitution, oxygen minimum zone

Deleted: Diversity of

Deleted: i

Deleted: oxygen minimum zone

Deleted: Biogeochemical implications of

Abstract

Intact polar lipids (IPLs) are the main building blocks of cellular membranes and contain chemotaxonomic, ecophysiologic and metabolic information, making them valuable biomarkers in microbial ecology and biogeochemistry. This study investigates IPLs in suspended particulate matter (SPM) in the water column of the Eastern Tropical North Pacific Ocean (ETNP), one of the most extensive open ocean oxygen minimum zones (OMZ) in the world with strong gradients of nutrients, temperature and redox conditions. A wide structural variety in polar lipid head group composition and core structures exists along physical and geochemical gradients within the water column, from the oxygenated photic zone to the aphotic OMZ. We use this structural diversity in IPLs to evaluate the ecology and ecophysiological adaptations that affect organisms inhabiting the water column, especially the mid-depth OMZ in the context of biogeochemical cycles. Diacylglycerol phospholipids are present at all depths, but exhibit highest relative abundance and compositional variety (including mixed acyl/ether core structures) in the upper and core OMZ where prokaryotic biomass was enriched. Surface ocean SPM is dominated by diacylglycerol glycolipids that are found in photosynthetic membranes. These and other glycolipids with varying core structures composed of ceramides and hydroxylated fatty acids are also detected with varying relative abundances in the OMZ and deep oxycline, signifying additional non-phototrophic bacterial sources for these lipids. Betaine lipids (with zero or multiple hydroxylations in the core structures) that are typically assigned to microalgae are found throughout the water column down to the deep oxycline but do not show a depth-related trend in relative abundance. Archaeal IPLs comprised of glycosidic and mixed glycosidic-phosphatidic glycerol dibiphytanyl glycerol tetraethers (GDGTs) are most abundant in the upper OMZ where nitrate maxima point to ammonium oxidation, but

Deleted: OMZ

Deleted: microbial

increase in relative abundance in the core OMZ and deep oxycline. Abundant non-phosphorus "substitute" lipids within the OMZ suggest that the indigenous microbes might be phosphorus limited (P starved) at ambient phosphate concentrations of 1 to 3.5 μM, although specific microbial sources for many of these lipids still remain unknown.

1. Introduction

Oxygen Minimum Zones (OMZ) are permanently oxygen-deficient regions in the ocean defined by O_2 concentrations <20 μM. They occur in areas where coastal or open ocean upwelling of cold, nutrient-rich waters drive elevated levels of primary production and the subsequent respiration of organic matter exported out of productive surface waters consumes oxygen faster than it is replaced by ventilation or by mid-depth lateral injections of oxygenated water. Low oxygen levels cause habitat compression, whereby species intolerant to low levels of oxygen are restricted to oxygenated surface waters (Keeling et al., 2010; Rush et al., 2012). But even these low levels of oxygen permit vertical migration of some zooplankton taxa into hypoxic waters (e.g., Seibel, 2011; Wishner et al., 2013). Oxygen depletion stimulates diverse microbial life capable of utilizing alternative electron acceptors for respiration under microaerobic conditions (e.g., Ulloa et al., 2012; Tiano et al., 2014; Carolan et al., 2015; Kalvelage et al., 2015; Duret et al., 2015). Important prokaryote-mediated processes within OMZs include denitrification and the anaerobic oxidation of ammonium (anammox), which together may account for 30-50% of the total nitrogen loss from the ocean to the atmosphere (Gruber, 2008; Lam and Kuypers, 2011). Modern day OMZs comprise ~8% of global ocean volume (Karstensen et al., 2008; Paulmier and Ruiz-Pino, 2009; Lam and Kuypers, 2011), but any expansion in the coming decades as a consequence of global warming and increased stratification (Stramma et al., 2008; Keeling et al., 2010) would have profound effects on marine ecology, oceanic productivity, global carbon and nitrogen cycles, the biological pump and sequestration of carbon (Karstensen et al., 2008; Stramma et al., 2010; Wright et al., 2012). A better understanding of the effect of low-O_2 on marine biogeochemistry and microbial ecology is thus warranted.

The Eastern Tropical North Pacific Ocean (ETNP), situated off the west coast of Mexico and Central America, hosts one of the largest OMZs in the open ocean, extending halfway across the Pacific Ocean and comprising ~41% of global OMZs (Lavín and Fiedler, 2006; Fiedler and Talley, 2006; Paulmier and Ruiz-Pino, 2009). By comparison, OMZs of the Eastern Tropical South Pacific Ocean off Peru and Chile and in the Arabian Sea are ~14% and ~8%, respectively, of global OMZs. In the ETNP, a sharp permanent pycnocline develops where warm, saline surface waters lie on top of a shallow thermocline, producing a highly stratified water column. Moderate primary production, dominated by picoplankton, depends on oceanic upwelling and wind mixing of coastal waters but is generally limited by the lack of micronutrient dissolved iron (Franck et al., 2005; Pennington et al., 2006). Remineralization, ~70% of which is microbially mediated (Cavan et al., 2017), of particulate organic carbon exported out of surface waters consumes oxygen at rates that cannot be balanced by ventilation across the pycnocline and by sluggish lateral circulation, leading to O_2 levels <2 μM at depths between ~100 and ~800 m. Abundances of micro- (Olson and Daly, 2013) and macro-zooplankton (Wishner et al., 2013; Williams et al., 2014) that are high in surface waters are reduced in the OMZ, and those macrozooplankton that are diel vertical migrators survive in the OMZ with reduced metabolic rates (Maas et al., 2014; Cass and Daly, 2015). Microbial abundances and activities for both heterotrophic and chemoautotrophic metabolisms are high in both surface waters and within the OMZ, but again with reduced metabolic rates in the OMZ (Podlaska et al., 2012). A strong nutricline indicates microbial nitrogen cycling involving co-occurring nitrification, denitrification and anammox (Rush et al., 2012; Podlaska et al., 2012), perhaps contributing up to 45% of the global pelagic denitrification (Codispoti and Richards, 1976). Microbial communities are mainly comprised of proteobacteria, with increasing contributions of archaea in deeper waters. Yet, on

94 average ca. 50% of the prokaryotic communities within the OMZ of the ETNP remained uncharacterized
95 (Podlaska et al., 2012).

96 Intact polar lipids (IPLs) are the main building blocks of cellular membranes and may be used to
97 characterize abundance and physiology of aquatic microorganisms from all three domains of life. IPLs
98 represent a diverse range of molecular structures, including phosphatidyl, glycosidic, phospho-glycosidic,
99 and amino acid polar head groups linked to glyceryl-acyl and glyceryl-*O*-alkyl apolar moieties. IPL
100 distributions have been documented in surface waters of the Eastern Subtropical South Pacific (Van Mooy
101 and Fredricks, 2010), the Western North Atlantic Ocean (Van Mooy et al., 2006; 200; Popendorf et al.,
102 2011a), the South Pacific Ocean (Kharbush et al., 2016), the Mediterranean Sea (Popendorf, et al., 2011b),
103 the North Sea (Brandsma et al., 2012), lakes (Bale et al., 2016), the Western English Channel (White et
104 al., 2015) and throughout the water columns of stratified water bodies (Ertefai et al., 2008; Schubotz et
105 al., 2009; Wakeham et al., 2012; Pitcher et al., 2011; Xie et l., 2014; Basse et al., 2014; Sollai et al., 2015).
106 Surface waters are typically dominated by nine IPL classes. Three diacylglycerol glycolipids,
107 monoglycosyl (1G-), diglycosyl (2G-) and sulfoquinovosyl diacylglycerol (SQ-DAG), are main IPLs
108 found in all thylakoid membranes of phototrophs, including those of cyanobacteria (Siegenthaler et al.,
109 1998)[1]. Three betaine lipids, diacylglyceryl homoserine (DGTS), hydroxymethyl-trimethyl-*β*-alanine
110 (DGTA) and carboxy-*N*-hydroxymethyl-choline (DGCC), are also generally abundant. Betaine lipids
111 are widely distributed in lower plants and green algae (Dembitsky, 1996) and are thus usually assigned to

[1] Elsewhere in the literature 1G-DAG, 2G-DAG, and SQ-DAG are also termed MGDG, DGDG and SQDG. However, we have opted to retain the 1G-DAG, 2-DAG, etc. nomenclature as other IPLs discussed throughout also contain monoglycosyl- and diglycosyl-moieties (e.g., 1G-GDGT and 2G-GDGT). Likewise, we retain the nomenclature PC-DAG, PE-DAG, and PG-DAG for phospholipids elsewhere termed PC, PE, PG.

eukaryotic algae in the ocean (Popendorf, et al., 2011a), but DGTS was recently also found in bacteria when phosphorus is limited (Yao et al., 2015; Sebastian et al. 2016). Three common detected phospholipids are diacylglycerol phosphatidyl choline (PC-DAG; often simply referred to elsewhere as PC), phosphatidyl ethanolamine (PE-DAG, often PE), and phosphatidyl glycerol (PG-DAG, often PG), all of which have mixed eukaryotic or bacterial sources in the upper water column (Sohlenkamp et al., 2003; Popendorf, et al., 2011a). Microbial source assignments have been broadly confirmed by isotope labeling studies (Popendorf, et al., 2011a). In oxygen-deficient subsurface waters IPL distributions are more diverse and other phospholipids such as diacylglycerol phosphatidyl (*N*)-methylethanolamine (PME-DAG), phosphatidyl (*N,N*)-dimethylethanolamine (PDME-DAG) and diphosphatidyl glycerol (DPG) increase in abundance; these IPLs occur in a number of bacteria that may inhabit low oxygen environments (Schubotz et al., 2009; Wakeham et al., 2012). Dietherglycerol phospholipids and glycosidic ceramides with unidentified sources have also been detected (Schubotz et al., 2009; Wakeham et al., 2012), the latter have been recently observed to be abundant in phosphorus-limited diatoms (Hunter et al., 2018). IPLs that are unique to marine archaea are comprised of glycerol dialkyl glycerol tetraethers (GDGT) core lipids with various glycosidic, diglycosidic and mixed phospho-glyco polar head groups (e.g., Schouten et al., 2008; Pitcher et al., 2011; Zhu et al., 2016; Elling et al., 2017). Abundances of archaeal IP-GDGTs vary considerably with depth, but are typically elevated in zones of water column oxygen depletion, especially where ammonium oxidizing thaumarchaea are abundant (Pitcher et al., 2011; Schouten et al., 2012; Sollai et al., 2015).

IPL can also be indicators of metabolic and physiologic status. Many organisms remodel their IPL composition when faced with environmental stressors such as changes in pH, salinity, temperature or

availability of nutrients (Zhang and Rock, 2008; Van Mooy et al., 2009; Meador et al., 2014; Carini et al., 2015; Elling et al., 2015). Replacing phospholipids with non-phosphorus containing substitute lipids is an important mechanism when facing nutrient phosphate starvation in oligotrophic surface waters where phosphate concentrations may be as low as nanomolar levels. Cyanobacteria replace PG-DAG with SQ-DAG (Benning et al., 1993; Van Mooy et al., 2006) and microalgae and some bacteria replace PC-DAG with DGTS (Geiger et al., 1999; Van Mooy et al., 2009; Popendorf, et al., 2011b) due to their similar ionic charge at physiological pH. Heterotrophic marine bacteria can replace PE-DAG with either 1G-DAG or DGTS (Carini et al., 2015; Sebastian et al., 2016; Yao et al., 2015). Notably, substitute lipids are also biosynthesized under micromolar concentrations of phosphate (Bosak et al., 2016).

Here, we use IPL distributions in suspended particulate matter (SPM) to characterize eukaryotic, bacterial and archaeal communities inhabiting the water column of the ETNP. This study is an extension of that of Xie et al. (2014), which focused on the distribution of core and intact polar archaeal and bacterial tetraether lipids at two stations investigated here (stations 1 and 8). The water column of the ETNP comprises distinct biogeochemical zones based on oxygen concentrations and IPL distributions reflect the localized ecology. Abundant non-phosphorus substitute lipids within the core of the OMZ suggest phosphorus limitation of the source microorganisms even at micromolar concentrations of phosphate. Overall our results provide deeper insight into the broad community composition and the physiologic state of microorganisms inhabiting OMZs.

Deleted: OMZ

2. Methods

2.1 Sample collection and CTD data

155 Suspended particulate matter (SPM) samples were collected at four stations (distance to shore:
156 400~600 km; Fig 1) along a northwest-southeast transect (Station 1: 13° 01.87'N，104° 99.83'W; Station
157 2: 11° 99.96' N，101° 22.82' W; Station 5: 10° 68.94' N，96° 34.12' W; and Station 8: 8° 99.46'N，
158 90°00.18'W) in the ETNP during the R/V *Seward Johnson* cruise in November 2007 (R/V *Seward Johnson*
159 Cruise Scientists, 2007). Station 1 in the Tehuantepec Bowl is an area of relatively low primary
160 productivity (e.g., 0.05 mg Chl-*a*/m^2; (Fiedler and Talley, 2006; Pennington et al., 2006) whereas Station
161 8 in the Costa Rica Dome is moderately productive (1 mg Chl-*a*/m^2). All stations are characterized by a
162 strong thermocline/pycnocline/oxycline (at 20-50 m depths depending on location) and a profound and
163 thick OMZ (down to ~2 μM O_2 between ~300-800 m depth). Station 1 is a reoccupation of the Vertical
164 Transport and Exchange II/III site from the early 1980's (Lee and Cronin, 1984; Martin et al., 1987;
165 Wakeham and Canuel, 1988; Wakeham, 1987, 1989).
166 Seawater was filtered *in-situ* using submersible pumps (McLane Research Laboratories WTS-142
167 filtration systems) deployed on the conducting cable of the CTD/rosette that measured temperature,
168 conductivity, oxygen, fluorescence/chlorophyll-*a* and transmissivity during pump deployments and
169 during pumping. Filtered water volumes ranged between 130 and 1800 L (Suppl. Table 1). Pumps
170 were fitted with two-tier 142 mm diameter filter holders: a 53 μm mesh Nitex "prefiltration" screen to
171 remove larger eukaryotes and marine snow aggregates and a double-stacked tier of ashed glass fiber filters
172 (142 mm Gelman type A/E, nominal pore size 0.7 μm). IPL concentrations we report represent minimum
173 values to reflect potentially inefficient collection of 0.7 μm particles by GFFs. Since pore size of the
174 filters may also decrease during filtration the recovered material may vary dependent on filtration time.

Following pump recovery, GFF filters and Nitex screens were wrapped in pre-combusted foil and stored frozen at -20°C until extraction.

2.2 Elemental, pigment and nutrient analysis

Particulate organic carbon (POC) and total particulate nitrogen (TN) were measured on 14 mm-diameter subsamples of each glass fiber filter (GFF) prior to lipid extraction; therefore, POC and TN concentrations reported here are only for <53 µm material. The plugs were acidified in HCl vapor in a desiccator for 12 hours to remove inorganic carbon. Elemental analysis was performed with a ThermoFinnigan Flash EA Series 1112 interfaced to a ThermoFinnigan Delta V isotope ratio mass spectrometer at the Skidaway Institute Scientific Stable Isotope Laboratory. Organic carbon and nitrogen contents were calibrated against internal laboratory chitin powder standards which in turn had previously been cross-calibrated against USGS 40 and 41 international standards.

Chlorophyll-*a* (Chl-*a*) and pheopigment concentrations were measured on-board the ship (Olson and Daly, 2013). Seawater samples (100 – 500 ml) from CTD casts were filtered onto Whatman GF/F filters (0.7 µm) which were immediately extracted with 90% acetone. Fluorescence was measured with a Turner Designs 10AU fluorometer and Chl-*a* concentrations were determined after Parsons et al (1984). Post-cruise HPLC analysis of pigments in 100 − 500 ml seawater samples filtered onto Whatman GF/F (0.7 µm) filters were conducted at the College of Charleston Grice Marine Laboratory, Charleston, SC on a Hewlett Packard 1050 system (DiTullio and Geesey, 2002).

Seawater samples for nutrient analyses (NO_2^-, NO_3^{-2}, NH_4^+ and PO_4^{3-}) were collected directly from Niskin bottles into acid-washed, 30-mL high-density polyethylene (HDP) bottles. After three rinses,

196 bottles were filled to the shoulder, sealed, and frozen (−20°C). All frozen samples were transported to
197 the Oceanic Nutrient Laboratory at USF for analysis using a Technicon Autoanalyzer II.
198
199 *2.3 Lipid extraction and analysis of intact polar lipids*
200 Lipids associated with the <53 µm SPM on the GFFs were Soxhlet-extracted shortly after the
201 expedition in 2008 using dichloromethane:methanol (DCM:MeOH; 9:1 v/v) for 8 h. Extracted lipids
202 were partitioned into DCM against 5% NaCl solution and dried over Na_2SO_4. Total lipid extracts (TLEs)
203 were stored at -20°C. Soxhlet extractions, rather than for example microwave assisted Bligh-Dyer
204 extractions, were chosen at the time because it was the only feasible way to handle the double 142mm
205 filters. Extraction protocol surely can affect IPL distributions; as shown by Lengger et al. (2012) for
206 smaller sediment samples.
207 IPL analyses by high-performance liquid chromatography-mass spectrometry (HPLC-MS) were
208 carried out initially in 2010/2011 and again in 2015 as instrument protocols improved. In between these
209 analyses we did not observe a notable selective loss of IPL compounds, instead we were able to detect a
210 much larger suite of IPL structures due to improved detection and chromatographic separation techniques
211 (Wörmer et al., 2013). The confidence in these results are supported by the analysis of IPL standards
212 (Suppl. Table 2) that are stored at -20 °C over several years (fresh standard mixtures are typically prepared
213 every 2 to 3 years), which do not indicate degradation of any particular IPL over time (Suppl. Fig. 1).
214 The analysis in 2010/2011 focused on absolute concentrations of the major IPLs (for distinction between
215 major and minor IPLs see results section). Aliquots of the TLE were dissolved in DCM/methanol (5:1
216 v/v) for injection on a ThermoFinnigan Surveyor HPLC system coupled to a ThermoFinnigan LCQ

Deleted: More recent IPL analyses typically utilize less harsh modified Bligh-Dyer extraction procedures, however, we believe that our finding labile IPLs, such as hexose-phosphate-hexose GDGTs, indicates that our results are not compromised (cf. Lengger et al., 2012).

DecaXP Plus ion-trap MS via electrospray interface (HPLC-ESI-IT-MS^n) using conditions described previously (Sturt et al., 2004; Xie et al., 2014). Ten µL of a known TLE aliquot spiked with C_{19}-PC as internal standard was injected onto a LiChrosphere Diol-100 column (150 × 2.1 mm, 5 µm, Alltech, Germany) equipped with a guard column of the same packing material. Absolute IPL concentrations were determined in positive ionization mode with automated data-dependent fragmentation of the two most abundant base peak ions. Acyl moieties of glycolipids and aminolipids were identified via HPLC-IT-ESI-MS^2 experiments in positive ionization mode, whereas phospholipid side chain composition was analyzed in negative ionization mode. Details of mass spectral interpretation, and identification of fatty acid moieties are described in Sturt et al. (2004) and Schubotz et al. (2009) and are exemplified in Suppl. Table 3. HPLC-MS analysis is not able to differentiate between double bonds or rings, therefore in the subsequent text we will refer to double bond equivalents (DBE) to include both possibilities, similarly absolute chain length cannot be determined as branched and straight chain alkyl chains cannot be differentiated, therefore we report total carbon atom numbers for the alkyl side chains. Assignment of the betaine lipid DGTS was according to the retention time of the commercially available standard DGTS (Avanti Polar Lipids, USA). The isomer DGTA, which elutes at a different retention time due to its structural difference (e.g., Brandsma et al., 2012) was not observed in the HPLC-MS chromatograms. For all analyses, response factors of individual IPLs relative to the injection standard C_{19}-PC were determined using dilution series of commercially available standards (Suppl. Table 2).

Subsequent analyses in 2015 were used to obtain sum formulas and IPL structures based on exact masses in the MS1 and MS-MS experiments and to additionally provide data on minor lipids, which were below detection limit during the 2010/2011 ion trap analyses (for distinction between major and minor

lipids see results section). For these measurements absolute quantities could not be determined since the TLE had been used for other experiments and the information on TLE amounts used was unknown; therefore, these analyses are used to describe relative abundances. Analyses were performed on a Bruker maXis Plus ultra-high resolution quadrupole time-of-flight mass spectrometer (Q-TOF) with an ESI source coupled to a Dionex Ultimate 3000RS UHPLC. Separation of IPLs was achieved using a Waters Acquity UPLC BEH Amide column as described in Wörmer et al. (2013), which resulted in better chromatographic separation of compounds and higher sensitivity compared to the 2010/2011 analyses. Relative proportions of compounds were quantified taking the different response factors of IPL classes into account. Peak areas in extracted mass chromatograms were corrected with absolute response factors determined in dilution series of commercially available standards (Suppl. Table 2). Some ions assigned to either PE-AEG and PC-AEG could not be quantified individually due to co-elution of these compounds and were thus quantified as one group using the mean response factor of PE- and PC-DAG. For compound classes for which no standards were available, (e.g., PI-DAG, OL and the unknown aminolipids AL-I and AL-II) the relative responses could not be corrected for. Assuming these compounds may ionize similarly as structurally related IPLs, values may be off by a factor of 0.2 to 1.4, which is the maximum range of response factors observed for the standards.

2.4 Statistical analysis

Nonmetric multidimensional scaling (NMDS) analysis was used to illustrate the relationships among objects hidden in a complex data matrix (Rabinowitz, 1975) and was performed in the free software R (version 3.4.3, www.r-project.org/) with *metaMDS* (vegan library, version 2.4-6) as described by

Wakeham et al. (2012). The datasets of relative lipid distribution and variations in carbon number and double bond equivalents were standardized by Hellinger transformation using the function *decostand*, while for all other variables (environmental parameters, microbial groups) absolute numbers were used. The compositional dissimilarity was calculated by Euclidean distance measure. The resulting plot shows the distribution of lipids and sampling depths. Microbial groups and geochemical parameters were overlaid by function *envfit*. Lower stress is related to high quality of solution, and stress values ≤ 0.1 indicate results of good quality (Rabinowitz, 1975). Non-parametric Spearman Rank Order Correlation analysis was performed on combined data of environmental variables and IPL ratios and IPL relative abundances of all four stations using SigmaPlot 11.0 (Systat Software Inc., San Jose, USA).

3. Results

3.1 Biogeochemical setting

All along the transect, the thin mixed layer (upper ~20 m) was warm, ~25−28 °C, with oxygen concentrations approaching air saturation at ~200 µM (Fig. 2). The euphotic zone (1% of surface photosynthetically active radiation) generally ranged between 50 and 80 m depth. The thermocline was abrupt at ~20-50 m, where temperatures dropped to ~15−18 °C and oxygen decreased to ~20 µM. Temperatures stabilized by ~250−300 m depth at ~10−12 °C and oxygen levels were <2 µM; especially at Station 8 there were spatially and temporally variable oxygen intrusions into the upper portion of the OMZ. By ~600−800 m depth, a deep oxycline was observed where oxygen concentrations began to rise again to ~40 µM at temperatures of ~4 °C by 1250 m. For the purposes of this discussion, the water column of the ETNP was partitioned into four horizons based on oxygen content: an oxic epipelagic zone

down to the thermocline (0–50 m; 200 µM > O_2 > 20 µM); an upper OMZ (Station 1 and 8: 50–300 m, Station 5: 50 – 350 m, Station 2: 50–200 m; 20 µM > O_2 > 2 µM); the core OMZ (Station 1 and 8: 300–800 m, Station 5: 350 – 600 m Station 2: 200 – 600 m; O_2 < 2 µM); and a deep oxycline (Station 1 and 8 ≥ 800 m, Station 2 and 5 ≥ 600 m; O_2 > 2 µM) of rising O_2 levels (Fig. 1a). Note that sampling at stations 1 and 8 reached to 1250 m depth so SPM from >750 m depth best represents the deep oxycline.

Deleted: core OMZ and

Chl-*α* was highest in surface waters with maximum values of 1.8 µg/L at 10 m at station 5, was between 0.2 and 0.7 µg/L at station 1, 2 and 8 and decreased to values close to zero below 100 m at all stations (Fig. 2; see also Fiedler and Talley, 2006, and Pennington et al., 2006, for additional results from previous surveys). HPLC analysis of accessory pigments (Goericke et al., 2000; Ma et al., 2009) showed that picoplankton, primarily *Prochlorococcus* (indicated by divinyl chlorophyll *α*), were an important component of the photoautotrophic community, along with diatoms (fucoxanthin), especially *Rhizosolenia* at the deep fluorescence maximum at stations 1 and 5 but *Chaetoceros* at station 8, and prymnesiophytes (19'hexanoyloxyfucoxanthin and 19'butanoyloxyfucoxanthin; DiTullio and Geesey, 2002; Suppl. Table 4). High phaeopigment abundances (up to 90% of [Chl-*α* + phaeopigments]) attested to algal senescence or grazing by macro- (Wishner et al., 2013; Williams et al. 2014) and micro-zooplankton (Olson and Daly, 2013) above and into the oxycline. Primary maxima in transmissivity corresponded with the peak Chl-*α* concentrations and fluorescence maxima, but secondary transmissivity maxima between 300 and 400 m at stations 1, 5, and 8 indicated elevated particle abundances in the core of the OMZ (Fig. 2).

Nitrite (NO_2^-) maxima in the OMZ at all stations coincided with nitrate (NO_3^{2-}) deficits (Fig. 3). Ammonium (NH_4^+) concentrations changed little through the water column (Fig. 3). Phosphate (PO_4^{3-}; Fig. 3) and total dissolved nitrogen (TDN; not shown) were low (respectively, < 0.5 and < 3 µM) in the

upper 20 m of the oxic zone, but increased in the OMZ. High PO_4^{3-} (up to 3.4 µM) and high TDN (up to 44.5 µM) were observed in the deep OMZ at stations 2, 5 and 8 (Fig. 3). N:P ratios were lower than the Redfield ratio (16) at all sites and depths (Fig. 3); N:P minima were lowest in surface waters (2.6 to 10 in the upper 20 m) and at ~500 m within the core OMZ and the deep oxycline at station 1 (<9).

POC and TN concentrations (< 53 µm material) were highest in the euphotic zone (POC: 20 – 100 µg/L; TN: 4 – 15 µg/L), rapidly dropping to 5 µg/L and 1 µg/L below the upper OMZ, respectively (Fig. 2; Suppl. Fig. 2). Secondary maxima for POC (~10 µg/L) and TN (~2 µg/L) within the core of the OMZ might reflect elevated microbial biomass there. Concentrations dropped in the deep oxycline to ≤3 µg/L and ≤0.5 µg/L for POC and TN, respectively.

Absolute IPL concentrations were determined by ion trap LCMS and varied between 250 and 1500 ng/L in the oxic zone and abruptly decreased more than 10-fold (to <20 ng/L) in the upper OMZ (Fig. 2). Secondary maxima in IPL concentrations (15–40 ng/L) within the OMZ at all stations roughly coincided with elevated numbers of prokaryotes (Fig. 2). IPL:POC ratios decreased with increasing depth (Fig. 2), tracking trends of POC, TN and IPL concentrations.

3.2 Changes in IPL composition with water column depth in the ETNP

In total, 24 IPL classes were identified in the ETNP (Fig. 4, Suppl. Fig. 3). Eleven major and thirteen minor IPL classes were detected in the QTOF analyses, which were classified according to their relative abundance: if an individual IPL comprised more than 10% of total IPLs at any depth of the four stations it was classified as a major IPL, compounds <10% were minor IPLs. Based on their head group composition IPLs were grouped into glycolipids, phospholipids or aminolipids. Figure 3 shows changes

Deleted: 1

Deleted: 2

in the relative abundances (as percentages of total IPLs, excluding isoprenoidal archaeal IPLs) of glycolipids, phospholipids and aminolipids as well as several substitute lipid ratios, reflecting preferential biosynthesis of non-phosphorus lipids to replace phospholipids under phosphate-limiting growth (cf. Van Mooy et al., 2006; Popendorf, et al., 2011b; Carini et al., 2015; Bosak et al., 2016). Relative abundances of non-isoprenoidal phospholipids were highest in the core OMZ between 400 and 600 m at all sites, where they comprise up to 45−76% at stations 1, 2 and 5 and between 12 and 61% at station 8. Phospholipid abundances were lower within the upper OMZ and oxic zone at all stations (between 4 and 55%) and in the deep oxycline at station 8 (<1%). Aminolipid content was highest in SPM from the upper 55 m at station 5 and 8 (10 to 25%), the core OMZ at station 8 (15 to 34%) and the deep oxycline at station 1 (17%). Lower aminolipid contents (2 to 11%) were observed in the oxic zone and the core OMZ at stations 1 and 2, the upper OMZ at station 5 (0 to 11%) and the deep oxycline at station 8 (<2%). Glycolipid abundance was >9% at all depths, with highest abundance (average 54%, max. 82%) within the upper OMZ and oxic zone at all stations and the deep oxycline at station 8. Values down to 9% were observed within the core OMZ.

3.2.1 Major lipids

The eleven major IPL classes included three IP-GDTs of archaeal origin: (1G-GDGT, 2G-GDGT and HPH-GDGT); and eight IPLs assigned to either a bacterial or eukaryotic origin: three glycolipids (1G-DAG, 2G-DAG, SQ-DAG), four phospholipids (PG-DAG, PE-DAG, PC-DAG, PE+PC-AEG) and one aminolipid (DGTS). All major lipid classes were found at almost all depths at all four stations, but with varying relative abundances (as % of total IPL; Fig. 4, Suppl. Table 1).

Archaeal IP-GDGTs: Relative abundances of archaeal IPL (IP-GDGTs) generally increased with depth from non-detectable in surface waters to >50% of total IPLs at station 8 (bottom of core OMZ and deep oxycline). Archaeal IP-GDGT abundances at stations 1 and 2 peaked at 30% (bottom of upper OMZ, core OMZ and deep oxycline) but were generally <10% at station 5 (Fig. 4). At station 1 and 2, 1G-GDGT and 2G-GDGT were most abundant with variable amounts of HPH-GDGTs, whereas 1G-GDGT and HPH-GDGT dominated archaeal IPLs at station 5 and 8 at most depths. Distributions of glycosidic IPL-GDGTs obtained in the present investigation corroborate the absolute values reported by (Xie et al., 2014) for stations 1 and 8: 1G-GDGT was more abundant than 2G-GDGT at station 8 when compared to station 1. The core GDGTs of 1G-GDGTs and HPH-GDGTs are dominated by GDGT-0 and crenarchaeol (Suppl. Fig. 4), whereas 2G-GDGTs are dominated by GDGT-2 and a small amount of crenarchaeol (Zhu et al., 2016)

Diacylglycerol lipids: The oxic zone and the upper OMZ were dominated (~50−80% of IPL) at all sites by the diacylglycerol glycolipids, 1G-DAG, 2G-DAG and SQ-DAG (Fig. 4). In the core OMZ and deep oxycline, relative amounts of 2G-DAG and SQ-DAG decreased to 4% and 12%, respectively. 1G-DAG abundances were lowest in the core OMZ at all stations, but were up to 47% of total IPL in the deep oxycline. Diacylglycerol phospholipids, PE-, PG- and PC-DAG, were the second most abundant IPLs. Abundances of PE- and PG-DAG were highest within the upper and core OMZ, constituting >50% in the core OMZ at station 1, >30% at stations 2 and 5, and 16% at station 8. PC-DAG, with average abundances of 5% at stations 1, 2, 8 and 3% at station 5, did not exhibit depth-related trends. The third most abundant diacylglycerol class was the betaine lipid DGTS, which was present throughout the water column at average abundances of 7% at station 1, 2 and 8, and 5% at station 5.

Deleted: 3

373 Major diacylglycerol lipids showed changes in average number of carbon atoms and double bond
374 equivalents (DBE) with depth (Fig. 5, Suppl. Table 5). The glycolipids and PC-DAG decreased in average
375 carbon number by up to three carbons and decreased in DBE by up to 2 at the top of the upper OMZ and
376 within the core OMZ compared to the oxic zone and the deep oxycline. Average carbon numbers for
377 PE- and PG-DAG and DGTS showed an inverse trend, both generally increasing up to two carbons
378 between the upper OMZ and the core OMZ. Changes in DBE were not as pronounced for PG-DAG and
379 DGTS, on average 1 to 2 DBE greater in surface waters than in deeper waters, while the number of DBE
380 increased on average with depth for PE-DAG.
381 *Acyl-ether glycerol lipids*: Mixed ether-ester glycerol core structures with either PE or PC head
382 groups were observed at all stations and all depths (generally 4-12%) except for the deep oxycline at
383 station 8.
384
385 *3.2.2 Minor lipids*
386 Thirteen minor IPL classes were identified, five of which were glycolipids, four phospholipids and
387 four aminolipids. All minor lipid classes were detected at each site except for OH-DGTS which was
388 absent at station 1. Some minor lipids were found at all depths, whereas others were restricted to specific
389 depth zones as defined by oxygen content (Fig. 4).
390 *Diacylglycerol lipids:* Two minor diacylglycerol glycolipids, 1G-OH-DAG and 3G-DAG, were
391 most abundant within the oxic zone and the upper OMZ, comprising between 2 to 15% of minor lipids on
392 average (0.1 to 0.6% of total IPLs), but were only sporadically found within the core OMZ and deep
393 oxycline. 1G-OH-DAG showed highest relative abundances at station 5, constituting up to 40% of minor

lipids. Four additional phospholipids with diacylglycerol core structures with the following head groups were identified: diphosphatidylglycerol (DPG), phosphatidyl-(*N*)-methylethanolamine (PME), phosphatidyl-(*N,N*)-dimethylethanolamine (PDME) and phosphatidyl inositol (PI). DPG, PME-DAG and PDME-DAG had highest relative abundances (respectively 65, 56 and 35% of minor IPL) within the upper and core OMZ, but at lower abundances within the oxic zone at all stations and in the deep oxycline at stations 1, 2 and 5. PI-DAG was most abundant in the oxic zone and the upper OMZ (up to 25% of minor IPL), but was also present in the core OMZ and the deep oxycline, except for station 8. Three types of aminolipids were observed as minor lipids. OH-DGTS with up to three hydroxyl-groups attached to the fatty acyl side chains (Suppl. Fig. 5) was observed at most depths at station 8 with an average relative abundance of 23% among the minor lipids; it was also occasionally detected at stations 2 and 5 within the oxic zone and upper OMZ. Two additional aminolipids had an undefined head group that exhibited fragmentation patterns characteristic of betaine lipids, but without established betaine head group fragments (Suppl. Fig. 6b, c). The tentatively assigned sum formula for the head group of the first unknown aminolipid (AL-I) at ca. 6.7 minutes LC retention time was $C_8H_{17}NO_3$ and for the second unknown aminolipid (AL-II) at 10.5 minutes was $C_7H_{15}NO_3$. The head group sum formula for AL-II matches that of DGCC, but the diagnostic head group fragment of m/z 252 was not detected, and furthermore, AL-II did not elute at the expected earlier retention time for DGCC. AL-I and AL-II were detected at most depths at all four stations, with average abundances of 1 to 6% of the minor lipids for AL-I and comparably higher relative abundances ranging from 16 to 36% for AL-II.

Acyl-ether glycerol lipid: One minor compound that eluted slightly earlier than SQ-DAG had a fragmentation pattern similar to SQ-DAG but with exact masses of the parent ion and MS-MS fragments

Deleted: 4

Deleted: 5

in both positive and negative ion mode that suggested a mixed acyl-ether glycerol core lipid structure (Suppl. Fig. 6 d, e). Tentatively assigned as SQ-AEG, this IPL was observed at most depths at all four stations with highest relative abundances of 5 to 60% of minor IPLs within the oxic zone.

Sphingolipids: Two types of sphingolipids were identified, monoglycosyl ceramide (1G-CER), and hydroxylated monoglycosyl ceramide (1G-OH-CER) with up to two hydroxyl groups attached to the hydrophobic side chains (Suppl Fig. 5e). Both were observed at all depths at stations 1, 2, and 5 at average relative abundances between 3 and 8% of minor IPLs, but neither was detected in the deeper part of the core OMZ or deep oxycline at station 8.

Ornithine lipids: Trace amounts (<4%) of ornithine lipids were detected in the core OMZ of stations 2 and 5.

3.2.3 Statistical relationships between environmental parameters and lipid distribution

Spearman Rank Order Correlation was used to evaluate relationships between relative lipid abundance of lipid classes and environmental parameters (Table 1). The glycolipids 2G- and SQ-DAG showed highly significant ($p<0.001$) and positive correlations with depth, fluorescence, POC, TN, temperature and Chl-α, significant positive correlations were also observed with oxygen. Both also showed highly significant but negative correlations with phosphate and nitrate, and these overall trends were mirrored in the SQ-DAG:PG-DAG ratio. Total glycolipids (GL) and 1G-DAG only showed correlations with a few environmental parameters and total GL were only significantly positively correlated with oxygen. Most aminolipids and phospholipids did not show significant correlations with environmental parameters and any other correlations were neither strongly positive nor negative.

Deleted: 5

Deleted: 3

Relative abundances of total aminolipids and aminolipid (AL) to phospholipid (PL) ratios correlated positively with ammonium. AL:PL also correlated positively with oxygen. Relative abundance of total phospholipids and most individual phospholipids (PG-, PE-, PME-, and PDME-DAG) correlated negatively with oxygen. The only phospholipid that significantly correlated with phosphate was PDME, however, the positive correlation is not strong (r^2<0.4).

NMDS analysis revealed that all samples from the oxic zone had a negative loading on the NMDS2 axis along with environmental variables such as oxygen, fluorescence, TN, POC and Chl-*α*. IPLs with a strong negative loading on the NMDS2 axis (<-0.2) were 1G-OH-DAG, SQ-AEG, 2G-DAG, SQ-DAG, PI-DAG and OH-DGTS. Most samples from the core OMZ and deep oxycline had a positive loading on the NMDS2 axis, together with depth, phosphate and nitrate. IPLs that showed a strong positive loading on the NMDS2 axis (>0.2) were PDME-DAG, 2G-GDGT, DPG, PME-DAG and HPH-GDGT. Almost all environmental variables had low *p*-values (<0.001), indicating highly significant fitted vectors with the exception of temperature, salinity, ammonium and nitrate. Highest goodness of fit statistic was observed with oxygen (r^2=0.54), followed by phosphate (r^2=0.48) and then fluorescence (r^2=0.46).

4. Discussion

The moderate primary productivity in surface waters of the ETNP, intense microbial degradation of particulate organic matter exported to the thermocline, and restricted midwater oxygen replenishment produce the strong, shallow (~20 m deep) oxycline and a ~500 m thick OMZ with dissolved oxygen concentrations of <2 μM, not unlike other oceanic OMZs (e.g., Ulloa et al., 2012). The ETNP is dominated by picoplankton, and micro-grazers reported consuming most phytoplankton production

461 (Landry et al., 2011; Olsen and Daly, 2013). Peak macrozooplankton biomass was located at the
462 thermocline, near the upper boundary of the OMZ, but a secondary biomass peak of a different
463 zooplankton assemblage was present at the deep oxycline once O_2 concentrations rose to ~2 μM (Wishner
464 et al., 2013). Shallow-water, plankton-derived particulate organic carbon is the primary food source for
465 zooplankton in the mixed layer, upper oxycline and core OMZ, whereas deep POC, some of which might
466 have been produced by microbes in the OMZ, is important for deep oxycline zooplankton (Williams et al.,
467 2014). Microbial community structure and activities are typical of other OMZs (Taylor et al., 2001; Lin
468 et al., 2006; Woebken et al., 2007; Wakeham et al., 2007; 2012). Cell numbers of total prokaryotes were
469 highest in the euphotic layer and decreased with depth at the thermocline but rose again within the core
470 OMZ (Podlaska et al., 2012). Elevated rates of chemoautotrophy, measured by dark dissolved inorganic
471 carbon (DIC) assimilation, were observed at several depths in the OMZ and in the lower oxycline.
472 Transfer of chemoautotrophically-fixed carbon into zooplankton food webs is also evident (Williams et
473 al., 2014). Bacteria dominate the prokaryotic community at all stations. Nitrifying bacteria constituted
474 3-7% of total DAPI-positive prokaryotes in surface waters; sulfate-reducing bacteria (17 and 34% of total
475 prokaryotes), planctomycetes (up to 24% of total prokaryotes), and anammox bacteria (<1% of
476 prokaryotes) in the upper OMZ and deep oxycline might be associated with anoxic microzones within
477 particle aggregates even at low dissolved oxygen concentrations (Woebken et al., 2007; Carolan et al.,
478 2015). Archaeal cell abundances peaked at the start of the upper OMZ at all stations (up to 37% of total
479 prokaryotes at station 2), within the core OMZ at station 2 (up to 54% of total detected cells) and within
480 the deep oxycline at station 5 and 8 (around 25%; Fig. 2e). Crenarchaeota/thaumarchaeota represented
481 ~20% of prokaryotes throughout the water column, generally being highest in the lower OMZ and deep

oxycline, and at stations 2 and 5 just above the secondary Chl-*a* maxima at ~75 m. Euryarchaeota were 16-20% of total prokaryotes, especially in waters above the OMZ.

Total IPL concentrations that were over 50 times higher in the surface waters than at deeper depths coincided with high Chl-*α* concentrations, reflecting the importance of phototrophic sources to the IPL pool above the thermocline. Below the thermocline, IPL concentrations generally track trends in microbial cell abundances, and elevated IPL concentrations in the upper and core OMZ coincide with elevated nitrite concentrations. The rapid decrease in IPL concentrations below ~100 m probably results from a combination of a dearth of potential source organisms and the decomposition of sinking detrital lipids (Harvey et al., 1986; Matos and Pham-Thi, 2009). IPL concentration decreases below the euphotic zone are well established (Van Mooy et al., 2006; Schubotz et al., 2009; Van Mooy and Fredricks, 2010; Popendorf et al., 2011b; Wakeham et al., 2012). We believe that the diverse molecular compositions and shifts in relative abundances of IPLs with changing geochemistry reflect a complex biological community structure and their ecophysiologic adaptation throughout the water column.

4.1 Provenance of IPLs in the ETNP

Variations in IPL distributions and head group and core lipid compositions reflect the biogeochemical stratification of the water column. Below we discuss potential sources of and possible physiological roles for IPLs in the different zones.

Deleted: oxygen-driven

4.1.1 Oxic zone

The glycosyldiacylglycerides that dominate the IPL composition in oxic surface waters, 1G-DAG,

2G-DAG and SQ-DAG, are major constituents of photosynthetic thylakoid and chloroplast membranes (Wada and Murata, 1998; Siegenthaler, 1998) and are therefore generally assigned to photosynthetic algae or cyanobacteria (Van Mooy et al., 2006; Popendorf et al., 2011b). These are also the likely predominant sources in our study, however, notably 1G-DAG may also be synthesized by heterotrophic bacteria (Popendorf et al., 2011a; Carini et al., 2015; Sebastian et al., 2016). In the oxic zone, 1G- and 2G-DAG are predominantly comprised of C_{16} and C_{18} fatty acids with zero to 5 double bond equivalents polyunsaturated acid (PUFA) combinations such as $C_{16:4}/C_{18:3}$, $C_{16:4}/C_{18:4}$, $C_{18:3}/C_{16:2}$, $C_{18:4}/C_{14:0}$ and $C_{18:5}/C_{14:0}$ (Suppl. Table 5, Fig. 5). These are characteristic of eukaryotic algae (Brett and Müller-Navarra, 1997; Okuyama et al., 1993), such as diatoms and prymnesiophytes that are the major eukaryotic phytoplankton in the ETNP. SQ-DAG biosynthesized by cyanobacteria do not contain PUFA, but instead predominantly contain combinations of $C_{14:0}$, $C_{16:0}$, and $C_{16:1}$ fatty acids (e.g., Siegenthaler, 1998), yielding shorter chain lengths and a lower average number of double bonds (0.5 to 1) than the other glycolipids as observed at the ETNP (Fig. 5). Betaine lipids (DGTS) in surface waters of the ETNP are comprised of C_{14}, C_{16}, C_{18} and C_{20} with multiple unsaturations or rings (on average 1.5 to 3 double bond equivalents) and are also likely phytoplankton derived (Dembitsky, 1996; Popendorf et al., 2011a).

PC-DAG with fatty acyl combinations of $C_{22:6}$ and $C_{20:5}$ long-chain PUFA and $C_{16:0}$ fatty acids (Suppl. Table 5) in surface waters also point to primarily eukaryotic algal sources. PG-DAG is the only phospholipid in cyanobacteria and thylakoid membranes of eukaryotic phototrophs (Wada and Murata, 1998). Heterotrophic bacteria are an additional source for PG-DAG since it can be a major phospholipid in bacterial membranes (Goldfine, 1984). PE-DAG is a minor phospholipid in eukaryotic algae (e.g., Dembitsky et al., 1996) but is common in membranes of bacteria (Oliver and Colwell, 1973; Goldfine,

1984) and is biosynthesized by heterotrophic marine bacteria (Popendorf et al., 2011a). Lower average number of double bond equivalents in PG- and PE-DAG (<2) in the upper water column of the ETNP are consistent with a bacterial origin (Fig. 5).

Oxic ETNP waters contain PE- and PC-based phospholipids with mixed acyl and ether core lipids (AEG), which are often referred to as 1-*O*-monoalkyl glycerol ethers (MAGE) if detected as core lipids. PE-AEG have been described in some sulfate-reducing bacteria (Rütters et al., 2001), which in the oxic zone or OMZ of the ETNP would require anoxic microzones in fecal pellets or aggregates (e.g., Bianchi et al., 1992; Shanks and Reeder, 1993). In the ETNP, MAGE-based phospholipids were 1 to 30% of total IPLs. MAGE, detected as core lipids in surface waters of the Southern Ocean and eastern South Atlantic are thought to be breakdown products of IP-AEGs of aerobic bacterial origin (Hernandez-Sanchez et al., 2014), but culturing experiments have yet to confirm this conclusion. Similarly, aerobic bacteria (possibly cyanobacteria) are likely sources for SQ-AEG, since sulfoquinovosyl is a diagnostic headgroup found in cyanobacteria, although, again, these lipids have not been reported in cultured cyanobacteria. Other minor phospholipids in the euphotic zone include PI-DAG and DPG. They are minor components in several marine algae (Dembitsky, 1996) and bacteria (Morita et al., 2010; Diervo et al., 1975; Mileykovskaya and Dowhan, 2009). Bacteria may also be the source of the low detected levels of *N*-methylated phospholipids PME-DAG and PDME-DAG (Goldfine and Ellis, 1964). 3G-DAG comprised of C_{14}, C_{16} and C_{18} fatty acids with up to six double bond equivalents is another minor IPL detected in the euphotic zone at all stations except for station 5. It has been found in some plants (Hölzl and Dörmann, 2007) and some anaerobic gram-positive bacteria (Exterkate and Veerkamp, 1969), which could both be probable sources in the oxic euphotic zone of the ETNP.

546 The sphingolipid, 1G-CER, consists of a sphingosine backbone linked to a fatty acid via an amide
547 bond and was a minor component in the oxic zone (<5% of IPL) at all stations (Fig. 4). Glycosidic
548 ceramides occur in eukaryotic algae such as the coccolithophore *Emiliania huxleyi* (Vardi et al., 2009).
549 We also detected 1G-OH-CER with up to 2 hydroxylations in the core lipid structure (Suppl. Fig. 5).
550 Multiple-hydroxylated sphingoid bases are potential markers of viral infection and cell death in at least
551 some marine phytoplankton, notably *E. huxleyi* (Vardi et al., 2009). We did not, however, find mass
552 spectral evidence for the presence of viral polyhydroxylated 1G-CER, as described by Vardi et al. (2009)
553 and therefore rather suggest that eukaryotic algal cells are potential sources for the 1G-CER (Lynch and
554 Dunn et al., 2004) in surface waters of the ETNP. We also detected hydroxylated glycolipids (1G-OH-
555 DAG) and aminolipids (OH-DGTS) with up to two hydroxyl-groups or one hydroxyl group combined
556 with an epoxy or keto function attached to the acyl groups (Suppl. Fig. 5). The addition of hydroxyl
557 groups or general oxidation of fatty acids in plants, algae and yeast is a defense mechanism and response
558 to oxidative stress (Kato et al., 1984; Andreou et al., 2009). Hydroxy fatty acids, for example, are
559 intermediates in oxidative degradation of fatty acids (Lehninger, 1970), and since they are constituents of
560 structural biopolymers of many microorganisms (Ratledge and Wilkinson, 1988), they are present in
561 marine particulate matter (e.g., Wakeham, 1999), likely derived from membrane constituents of Gram-
562 negative bacteria, the most abundant bacteria in seawater (Rappé and Giovannoni, 2000).
563
564 *4.1.2 Upper OMZ*
565 Glycolipid abundance varied between 15 to 80% of total IPL within the upper OMZ below the
566 thermocline/oxycline. SQ-DAG and 2G-DAG exhibited strong decreases in relative and absolute

Deleted: 4

Deleted: 4

abundance below 125 m at all stations consistent with the decrease in their phototrophic biomass. Number of carbon atoms in the core lipid chains and number of double bond equivalents of glycolipids showed considerable variations within the upper OMZ (Fig. 5), indicating a different assemblage of source organisms compared to the oxic zone. Likewise, decreasing carbon numbers and double bond equivalents for PC-DAG and DGTS combined with a dominance by C_{14}, C_{16} and C_{18} saturated and monounsaturated fatty acids (Suppl. Table 5) supports a shift from eukaryotic to bacterial sources. This suggests the diverse proteobacteria in the upper OMZ may biosynthesize non-phosphorus substitute IPLs. 1G-DAG or DGTS are known to replace phospholipids, primarily PE-DAG and PC-DAG under phosphorus limited growth (Geske et al., 2012; Carini et al., 2015; Sebastian et al., 2016; Yao et al., 2015), including at the phosphate concentrations of 2 to 2.5 μM in the upper OMZ. Sulfate-reducing proteobacteria, which comprise up to 10% of the total bacteria in the ETNP (Podlaska et al., 2012) may be candidate organisms for this phospholipid to glycolipid replacement (Bosak et al., 2016). Structures of minor IPLs, AL-I and AL-II were not fully elucidated (see Suppl. Fig. 6) and their origins remain uncertain. PME- and PDME-DAG, DPG, 1G-CER and 1G-OH-CER within the upper OMZ are consistent with previous reports of their production by (unidentified) bacteria near redox boundaries in other stratified water bodies (Schubotz et al., 2009; Wakeham et al., 2012).

Deleted: 5

Archaeal IPLs with glycosidic headgroups and tetraether core structures (1G- and 2G-GDGT) comprised a greater proportion of the overall IPL pool within the upper OMZ than in surface waters. Analysis of these same samples by Xie et al. (2014) first reported that concentrations of glycosidic GDGTs peak in the ETNP roughly at depths where nitrite maxima are observed. IP-GDGTs with the hexose-phosphate-hexose (HPH) headgroups and the core GDGT crenarchaeol (Suppl. Fig. 4) of thaumarchaeota

Deleted: 3

592 (Schouten et al., 2008; Elling et al., 2017) were most abundant at depths of nitrate maxima at all ETNP
593 stations, as they are in other oxygen-deficient water columns (e.g., Pitcher et al., 2011; Lengger et al.,
594 2012; Schouten et al., 2012; Sollai et al., 2015), although they were present at greater depths in the ENTP
595 as well. The microbial enumerations by Podlaska et al. (2012) had shown previously that
596 thaumarchaeota (referred to as crenarchaeota) and euryarchaeota constitute almost equal amounts to <10%
597 of total cell number in the upper OMZ of the ETNP. It is also possible that uncultured marine Group II
598 euryarchaeota are additional sources for glycosidic GDGTs as has been suggested previously (Lincoln et
599 al., 2014; Zhu et al., 2016).
600
601 *4.1.3 Core OMZ and deep oxycline*
602 IPL distributions in the core OMZ and at the deep oxycline of the ETNP that were notably different
603 from the oxic zone and the upper OMZ are consistent with *in-situ* microbial origins. We choose to
604 discuss the core OMZ and deep oxycline together because, although oxygen concentrations are beginning
605 to rise in the deep oxycline, IPL compositions in both zones are similar and likely reflect similar
606 biogeochemical sources. Phospholipid abundance at all stations generally increased to over 50% (except
607 for station 8) at the expense of glycolipids. PE and PG-DAG are the most abundant phospholipids in the
608 core OMZ, along with PC-DAG and PE- and PC-AEG, DPG. PME and PDME-DAG are all common
609 lipids in α-, γ- and some β-proteobacteria (Oliver and Colwell, 1973; Goldfine, 1984) that are present in
610 the OMZ (Podlaska et al., 2012). Changes in phospholipids chain length and number of double bond
611 equivalents further support *in-situ* IPL production (Fig. 5). Fatty acid combinations for phospholipids
612 were dominated by saturated $C_{14:0}$, $C_{15:0}$ and $C_{16:0}$ and monounsaturated $C_{16:0}$ C_{17} and $C_{18:0}$ (Suppl. Table

Deleted: Therefore, we conclude

Deleted: ,

Deleted: also potential

Deleted:

5); PUFA were generally of reduced abundance, and odd-numbered fatty acids increased in proportion. In the case of PUFA, even though they may be biosynthesized by piezophilic aerobic deep-sea bacteria (DeLong and Yayanos,1986, Fang et al. 2003; Valentine and Valentine, 2004), either the microaerophilic bacteria in the deep OMZ of the ETNP do not produce PUFA or these labile fatty acids are rapidly degraded *in-situ* (DeBaar et al., 1983; Prahl et al., 1984; Neal et al., 1986).

Among glycolipids, 1G-DAG was most abundant at the deep OMZ/oxycline at stations 1 and 8; here 1G-DAG abundance actually increases over that of shallower depths. Carbon number and number of double bond equivalents for glycolipids are again distinct from the surface waters, with on average 1 to 2 carbon atoms shorter chain lengths and 1 to 3 fewer double bonds (Fig. 5), supporting the notion that at least some of these glycolipids are biosynthesized *in-situ* and not simply exported from the surface waters. In particular, SQ-DAG in the core OMZ/oxycline contained odd-carbon numbered fatty acids (e.g., $C_{15:0}/C_{16:0}$ and $C_{14:0}/C_{15:0}$) different from the cyanobacterial SQ-DAG in surface waters (Suppl. Table 5). Some Gram-positive bacillus and firmicutes biosynthesize 1G, 2G- and SQ-DAG (Hölzl and Dörmann, 2007) and 1G-, 2G- and SQ-DAG in deeply buried Wadden Sea sediments are attributed to anaerobic bacteria (Seidel et al., 2012). However, Gram-positive bacteria are generally not abundant in seawater.

The core OMZ/deep oxycline are particularly enriched in archaeal GDGT, notably 1G-GDGT and HPH-GDGT, with predominantly GDGT-0 and crenarchaeol as core lipids (Suppl. Fig. 4). At stations 1 and 8 where sampling penetrated below ~800 m depth, 1G-GDGT and HPH-GDGT constitute up to ~60% and ~22%, respectively, of total IPL. Significantly, the elevated abundances of 1G-GDGT and HPH-GDGT at the bottoms of the sampling depth profiles in the deep oxycline of stations 1 and 8 correspond to depths at which ammonium concentrations are higher than shallower in the core OMZ (Fig. 2).

Deleted: 3

Remineralization at the deep-oxycline might provide additional ammonium to drive thaumarchaeotal ammonium oxidation and production of archaeal IPLs.

4.2 Factors influencing IPL distribution in the ENTP

4.2.1 Factors affecting structural diversity of the core lipid composition

IPL in the ETNP display considerable diversity not only in the headgroup but also core lipid types, from diacylglycerol lipids with varying number of carbon atoms (likely chain lengths) and zero to multiple double bond equivalents (likely reflecting the number of unsaturations), with or without hydroxylations to mixed ether/ester glycerolipids, sphingolipids and ornithine lipids. Statistical analysis provides aids in illuminating influences of environmental factors and microbial community structure on the lipid composition in the water column of the ETNP. Changes in core alkyl lipid chain length and degree of unsaturation are often associated with temperature (Neidleman, 1987), even at the range of temperatures of the ETNP water column. However, NMDS analysis did not yield any strong correlations between temperature and number of carbon atoms in the side chains or double bond equivalents of the major IPL classes ($r^2 < 0.02$, Suppl. Table 6), nor with other environmental parameters (r2 < 0.3, Suppl. Table 6). Instead, changing biological sources may play a decisive role in determining number of carbon atoms and double bond equivalents in the ETNP. For instance, long-chain PUFAs in surface waters are mainly synthesized by phytoplankton, while in deeper waters some bacteria may biosynthesize these PUFAs. The degree of hydroxylation in the acyl side chains also did not show any clear link to specific environmental factors, although, both 1G-OH-CER and OH-DGTS had negative loadings on the NMDS-2 axis indicating a higher abundance of these compounds in oxic samples. It is possible that hydroxylated

660 IPLs play a role during oxidative stress and/or are involved in other defense mechanisms (Kato et al.,
661 1984; Andreou et al., 2009).

662 Mixed ether-acyl lipids have been reported in various oceanic settings (Hernandez-Sanchez et al.,
663 2014). In our study, there was no noticeable correlation between PE- and PC-AEG and depth or oxygen
664 concentrations (Fig. 6). Ornithine lipids were strongly negatively loaded on the NMDS-1 axis, but none
665 of the measured environmental parameters could account for this negative loading (Fig. 6). Therefore,
666 it remains unclear what factor(s) ultimately determine their distribution. Likewise, there were no
667 significant correlations between the sphingolipid 1G-CER and any environmental parameter. Since
668 ether-acyl lipids, ornithine lipids and sphingolipids play many functional roles in biological systems, their
669 variable distribution within the water column reflect most likely the diversity of microbes inhabiting the
670 dynamic oxygen regime of the ETNP.

671

672 *4.2.2 Factors influencing head group composition*

673 NMDS analysis of normalized IPL composition and quantitative microbial data (abundance of α, β,
674 γ, ε-proteobacteria, sulfate-reducing bacteria δ-proteobacteria, planctomycetes, crenarchaeota including
675 thaumarchaeota and euryarchaeota) did not yield any high goodness of fit statistic ($r^2 < 0.3$; Suppl. Table
676 6) that would clearly delineate specific prokaryotic sources for the various IPL. This absence of
677 statistical correlation would result if neither the IPL compositions of SPM nor the structure and lipid
678 composition of the prokaryotic community were sufficiently unique to strongly distinguish the
679 biogeochemical zones. Indeed, although there are depth-related differences in IPL composition of SPM
680 and prokaryotic community, there is considerable overlap. Therefore, instead of trying to elucidate

specific IPL sources, we here query the affect environmental factors such as temperature, nutrient or oxygen concentrations may have on the IPL compositions in the ENTP, and by analogy to natural marine settings in general. Most the major and minor glycolipids were loaded negatively on the NMDS2 axis, as were oxygen, fluorescence, Chl-α, POC and TN (Fig. 6). A notable exception was 1G-DAG which had only a slightly negative loading on the NMDS-2 axis. These relationships (loadings) roughly reflect the vertical distribution of IPLs in the water column of the ETNP. Glycolipids, particularly 2G-DAG and SQ-DAG, were most abundant in the euphotic oxic zone characterized by high oxygen concentration and moderate primary productivity, dominated by phytoplankton, primarily cyanobacteria (high POC, TN and elevated Chl-α and fluorescence). Spearman Rank Order Correlations confirm these observations, including the lack of significant correlations between 1G-DAG and depth or any other environmental parameter. One explanation is that 1G-DAG originates from assorted sources throughout the water column independent of any single environmental variable. Similarly, PC-DAG, PG-DAG, and DGTS did not correlate with any of the tested environmental variables, because their compositions are relatively homogeneous across all biogeochemical zones. PE-, PME- and PDME-DAG, and DPG, on the other hand, that became more prevalent within the core OMZ, and at deeper depths where oxygen concentrations decrease and nutrient (NO_3^- and PO_4^{3-}) concentrations were elevated due to organic matter remineralization, gave positive loadings with these environmental parameters on the NDMS2 axis. Archaeal IPLs showed positive loadings on the NMDS2 axis, consistent with the increasing importance of archaeal abundance with depth and at reduced oxygen concentrations.

4.2.3 Links between substitute lipid ratios and nutrient concentrations

SQ-DAG and PC-DAG are often the most abundant respective glycolipids and phospholipids in the surface ocean (Popendorf et al., 2011a,b), including the Eastern Tropical South Pacific (Van Mooy and Fredricks, 2010). The abundance of SQ-DAG in the surface waters of the ETNP (18-50% of total IPL) is thus not unusual. In the ETNP, however, PC-DAG was comparably minor (3-13% of total IPL). Instead, DGTS was abundant at some stations, up to ~20% of major IPL at station 5. SQ-DAG and DGTS serve similar biochemical functions as the phospholipids PG-DAG and PC-DAG, respectively, due to similar ionic charges at physiological pH. The former may be preferentially biosynthesized by phytoplankton and some bacteria as substitute lipids for PG-DAG and PC-DAG when phosphate starved (Benning, 1993; Van Mooy et al., 2006, 2009). Likewise, 1G-DAG, glycuronic acid diacylglycerol (GADG) and ornithine lipids may substitute for PE-DAG in marine bacteria (e.g., chemoheterotrophic α-proteobacteria of the SAR11 clade of *Pelagibacter* sp.: Carini et al., 2015; the sulfate reducing bacterium, *Desulfovibrio alaskensis*: Bosak et al., 2016). In oligotrophic surface waters of the Sargasso Sea (PO_4^{3-} <10 nM) ratios of SQ-DAG:PG-DAG and DGTS:PC-DAG are high (4 to 13) compared to the same ratios (3) in the phosphate replete South Pacific (PO_4^{3-} >100 nM), consistent with cyanobacteria synthesizing phosphorus-free substitute lipids to maintain growth in response to phosphorus deprivation (Van Mooy et al., 2009). At the ETNP, SQ-DAG:PG-DAG ratios ranged between 1 and 10 within the upper 100-200 m along the transect and were <1 deeper into the OMZ (Fig. 3). DGTS:PC-DAG ratios in the ETNP were quite variable, ranging between 0.4 and 2.4 at most depths, but with notable spikes (>30) within the oxic zone at station 5, within the upper core OMZ at station 2 and 8 and in the lower portion of the core OMZ at station 8. 1G-DAG:PE-DAG ratios where highly variable (0.2 to 945) and were highest within the upper OMZ at station 2, 5 and 8 and within the deep oxycline at station 8, where 1G-DAG:PE ratios

range between 290 and 945 (Fig. 3). To test the substitute lipid hypothesis for the ETNP, we performed a Spearman Rank Order Correlation analysis of known substitute lipid ratios as well as total aminolipid (AL) to phospholipid (PL) and total glycolipid (GL) to PL ratios with nutrient concentrations and other environmental parameters. Only SQ-DAG:PG-DAG was significantly correlated with phosphate (-0.56, $p<0.001$) but also correlated with other parameters, such as depth (-0.76, $p<0.001$) and oxygen concentration (0.58, $p<0.001$). These correlations reflect the elevated SQ-DAG:PG-DAG ratios (2-8) in the surface waters and upper OMZ (Fig. 3) and support the notion that SQ-DAG might serve as a substitute lipid in both surface waters and the OMZ when phosphate concentrations are in the low micromolar range (~0.1-0.4 μM in surface waters; ~2-3.5 μM in the OMZ). Other proposed substitute lipid ratios, DGTS:PC-DAG (Van Mooy et al., 2009) and 1G-DAG:PE-DAG (Carini et al., 2015), did not correlate with nutrient concentrations in the water column of the ETNP but rather showed highly variable distributions. Similarly, AL:PL ratios did not exhibit strong relationships with any environmental parameter, and GL:PL ratios showed similar but less pronounced trends as SQ-DAG:PG-DAG ratios. Overall, we observed no correlation between these substitute lipid ratios and phosphate concentration in the ETNP. We propose that non-phosphorus IPL within the OMZ of the ETNP originate from bacteria growing under low micromolar concentrations of phosphate. Indeed, the culture experiments of Bosak et al. (2016) demonstrated that the sulfate reducer, *Desulfovibrio alaskensis*, begins to replace most of its membrane phospholipids with 1G-DAG, glycuronic acid diacylglycerol and ornithine lipids even at phosphate concentrations as high as 20 μM.

5. Conclusions

744 The water column of the ETNP is characterized by a diverse suite of intact polar lipids. IPL
745 distributions reflect the dynamic nature of the biological community in the ETNP, with light and oxygen
746 as primary determinants, from fully oxygenated euphotic surface waters to an aphotic strong oxygen
747 minimum zone at mid-depth. Highest concentrations of IPLs (250 – 1500 ng/L) in oxygenated surface
748 waters zone results from abundant phototrophic eukaryotic and cyanobacterial sources above the OMZ.
749 Secondary peaks in IPL concentration (12 – 56 ng/L) within the core of the OMZ mirror elevated
750 abundances of heterotrophic and chemoautotrophic bacteria and archaea under low oxygen conditions.
751 Glycolipids derived from photoautotrophs generally accounted for more than 50% of total IPLs in the
752 euphotic zone (< 200 m, oxic and upper OMZ zones), but bacterial phospholipids were more abundant
753 (avg. 40%) in the OMZ and deep oxycline layers. Archaeal GDGTs were abundant within the OMZ and
754 deep oxycline, consistent with elevated archaeal abundances there. Variations in major fatty acid
755 constituents within IPL classes with acyl core moieties show that biological source(s) for the different IPL
756 were distinct in each depth/oxygen-content horizon. Nevertheless, microbial sources for many of the
757 detected lipids remain unclear and therefore potentially unique ecophysiological adaptations these lipids
758 may represent remain to be explored.
759 The presence of the glycolipid, monoglycosyl diacylglycerol (1G-DAG), and the betaine lipid,
760 diacylglyceryl homoserine (DGTS), both with varying fatty acid compositions, within all biogeochemical
761 zones, and especially in the OMZ, indicates that these canonical phototrophic markers are not only
762 biosynthesized in surface waters, but may indeed be produced in the aphotic water column and by a much
763 larger host of organisms than previously thought. Since 1G-DAG and DGTS can be biosynthesized by
764 various bacteria to replace phospholipids under phosphorus limited growth, we suggest that they serve as

Deleted: a

non-phosphorus substitute lipids for some microorganisms in the OMZ. The presence of these substitute lipids at micromolar concentrations of phosphate of the ETNP suggests that the paradigm of substitute lipid biosynthesis being restricted to the PO_4^{3-}-depleted oligotrophic surface ocean may need to be re-evaluated.

Author contribution

SGW collected the samples. SGW, FS and KUH designed the study. SX and FS measured and processed the data. JSL and FS performed statistical analyses. FS and SGW wrote the paper with input from SX, KUH and JSL.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgments

We are grateful to the captain and the crew of R/V *Seward Johnson*, to K. Daly and K. Wishner as co-chief scientists, and to the U.S. National Science Foundation for supporting the cruise. H. Albrecht, B. Olsen and S. Habtes helped with PM sampling. We thank K. Fanning and R. Masserini (University of South Florida) for providing their nutrient results; C. Flagg (Stony Brook) processed CTD hydrographic data; Jay Brandes and Mary Richards (Skidaway Institute) conducted the POC and TN analyses; B. Olson and K. Daly (University of South Florida) provided ship-board Chl-*a* analyses; and G. DeTullio (College of Charleston) conducted HPLC analyses of pigments. Lab supplies and analytical infrastructure for

lipid analyses was funded by the Deutsche Forschungsgemeinschaft (DFG, Germany) through the Cluster of Excellence/Research Center MARUM. The UHPLC-QTOF instrument was granted by the DFG, Germany through grants Inst 144/300-1. S. Xie was funded by the China Scholarship Council, F. Schubotz by the Zentrale Forschungsförderung of the University of Bremen, and U.S. National Science Foundation grant OCE-0550654 to S. G. Wakeham supported this project. SGW also acknowledges a Fellowship from the Hanse-Wissenschaftskolleg (Hanse Institute for Advanced Studies) in Delmenhorst, Germany.

References

Andreou, A., Brodhun, F., Feussner, I.: Biosynthesis of oxylipins in non-mammals, Progr. Lip. Res., 48, 148-170, 2009.

Bale, N. J., Hopmans, E. C., Schoon, P. L., de Kluijver, A., Downing, J. A., Middelburg, J. J., Sinninghe Damsté, J. S. and Schouten, S.: Impact of trophic state on the distribution of intact polar lipids in surface waters of lakes. Limnol. Oceanogr., 61, 1065–1077, 2016.

Basse, A., Zhu, C., Versteegh, G.J.M., Fischer, G., Hinrichs, K.-U., and Mollenhauer, G.: Distribution of intact and core tetraether lipids in water column profiles of suspended particulate matter off Cape Blank, NW Africa, Org. Geochem., 72, 1-13, 2014.

Benning, C., Beatty, J. T., Prince, R. C., and Somerville C. R.: The sulfolipid sulfoquinovosyldiacylglycerol is not required for photosynthetic electron transport in Rhodobacter sphaeroides but enhances growth under phosphate limitation, Proc. Natl. Acad. Sci. USA, 90, 1561–1565, 1993.

Bianchi, M., Marty, D., Teyssié, J.-L., and Fowler, S. W.: Strictly aerobic and anaerobic bacteria associated with sinking particulate matter and zooplankton fecal pellets, Mar. Ecol. Press Ser., 88, 55-60, 1992.

Bosak, T., Schubotz, F., de Santiago-Torio, A., Kuehl, J. V., Carlson, H. K., Watson, N., Daye, M., Summons, R. E., Arkin, A. P., and Deutschbauer A. M.: System-wide adaptations of *Desulfovibrio alaskensis* G20 to phosphate-limited conditions, PLoS ONE 11, e0168719, 2016.

Brandsma, J., Hopmans, E. C., Philippart, C. J. M., Veldhuis, M. J. W., Schouten, S., and Sinninghe Damste, J. S.: Low temporal variation in the intact polar lipid composition of North Sea coastal marine water reveals limited chemotaxonomic value, Biogeosciences, 9, 1073–1084, 2012.

Brett, M. T., and Müller-Navarra, D. C.: The role of highly unsaturated fatty acids in aquatic foodweb pro- cesses, Freshw. Biol., 38, 483–499, 1997.

Carini P., Van Mooy B. A. S., Thrash J. C., White A., Zhao Y., Campbell E. O., Fredricks H. F., and Giovannoni S. J.: SAR11 lipid renovation in response to phosphate starvation. Proc. Natl. Acad. Sci. USA, 112, 7767–7772, 2015.

Carolan, M.T., Smith, J.M., and Beman, J.M.: Transcriptomic evidence for microbial sulfur cycling in the eastern tropical North Pacific oxygen minimum zone. Front. Microbiol. 6, 334, 2015.

Cass, C. J., and Daly, K. L.: Ecological characteristics of eucalanoid copepods of the eastern tropical North Pacific Ocean: Adaptations for life within a low oxygen system, J. Exp. Mar. Biol. Ecol., 468, 118-129, 2015.

Cavan, E. L., Trimmer, M., Shelley, F., Sanders, R.: Remineralization of particulate organic carbon in an ocean oxygen minimum zone, Nat. Comm., 8, 14847, 2016.

Codispoti, L. A., and Richards, F. A.: An analysis of the horizontal regime of denitrification in the eastern tropical North Pacific. Limnology and Oceanography 21, 379-388, 1976.

DeBaar, H. J. W., Farrington, J. W. and Wakeham, S. G.: Vertical flux of fatty acids in the North Atlantic Ocean, J. Mar. Res., 41, 19-41, 1983.

DeLong, E. F. and Yayanos, A.: Biochemical function and ecological significance of novel bacterial lipids in deep-sea procaryotes, Appl. Environ. Mirobiol., 51, 730-737, 1986.

Dembitsky, V.: Betaine ether-linked glycerolipids: Chemistry and biology, Progr. Lip. Res., 35, 1-51, 1996.

Diervo, A. J. and Reynolds, J. W.: Phospholipid composition and cardiolipin synthesis in fermentative and nonfermentative marine bacteria, J. Bacteriol. 123, 294-301, 1975.

DiTullio, G., and Geesey, M. E.: Photosynthetic Pigments in Marine Algae and Bacteria. In: G Bitton (ed), Encyclopedia of Environmental Microbiology, vol. 5, Wiley, pp 2453-2470, 2002.

Elling, F. J., Könneke, M., Mußmann, M., Greve, A., and Hinrichs, K.-U.: Influence of temperature, pH, and salinity on membrane lipid composition and TEX86 of marine planktonic thaumarchaeal isolates, Geochim. Cosmochim. Acta, 171, 238-255, 2015.

Elling, F. J., Könneke, M., Nicol, G. W., Stieglmeier, M., Bayer, B., Spieck, E., La Torre, De J. R., Becker, K. W., Thomm, M., Prosser, J. I., Herndl, G. J., Schleper, C., and Hinrichs, K.-U. Chemotaxonomic characterisation of the thaumarchaeal lipidome, Environ. Microbiol. 10, 1080, 2017.

Ertefai, T., Fisher, M., Fredricks, H. and Lipp, J.: Vertical distribution of microbial lipids and functional genes in chemically distinct layers of a highly polluted meromictic lake, Org. Geochem., 39, 1572-1588, 2008.

Exterkate, F. A., and Veerkamp, J. H.: Biochemical changes in Bifidobacterium bifidum var. Pennsylvanicus after cell wall inhibition. I. Composition of lipids, Biochim. Biophys. Acta, 176, 65–77, 1969.

Fang, J., Kato, C., Sato, T., Chan, O., and McKay, D.: Biosynthesis and dietary uptake of polyunsaturated fatty acids by piezophilic bacteria. Comp. Biochem. Physiology Part B, 137 455–46, 2004.

Fiedler, P. C., and Talley, L. D.: Hydrography of the eastern tropical Pacific: A review. Progr. Oceanogr., 69, 143-180, 2006.

Franck, V. M., Smith, G. J., Bruland, K. W., and Brzezinski, M. A.: Comparison of size-dependent carbon, nitrate and silicic acid uptake rates in high- and low-iron waters. Limnol. Oceanogr., 50, 825-838, 2005.

Geiger, O., González-Silva, N., López-Lara, I. M., and Sohlenkamp, C.: Amino acid-containing membrane lipids in bacteria, Progr. Lip. Res., 49, 46–60, 2010.

Geiger, O., Röhrs, V., Weissenmayer, B., Finan, T. M., and Thomas-Oates, J. E.: The regulator gene phoB mediates phosphate stress-controlled synthesis of the membrane lipid diacylglyceryl-N,N,N-trimethylhomoserine in Rhizobium (Sinorhizobium) meliloti, Mol. Microbiol., 32, 63–73, 1999.

Geske, T., Dorp vom, K., Dörmann, P., and Hölzl G.: Accumulation of glycolipids and other non-phosphorous lipids in Agrobacterium tumefaciens grown under phosphate deprivation, Glycobiol., 23, 69–80, 2012.

Goericke, R., Olson, R. J., and Shalapyonok, A.: A novel niche for Prochlorococcus sp. in low-light suboxic environments in the Arabian Sea and the Eastern Tropical North Pacific, Deep Sea Res. I, 47, 1183-1205, 2000.

871 Goldfine, H.: Bacterial membranes and lipid packing theory, J. Lip. Res., 25, 1501–1507, 1984.
872 Goldfine, H., and Ellis, M. E.: N-methyl groups in bacterial lipids, J. Bacteriol., 87, 8–15, 1964.
873 Gruber, N.: The marine nitrogen cycle: overview and challenges, in: Nitrogen in the marine environment,
874 Eds. DG Capone, DA Bronk, MR Mulholland, EJ Carpenter, Burlington, MA, USA: Academic, 1-50,
875 2008.
876 Harvey, R. H., Fallon R. D., and Patton, J. S.: The effect of organic matter and oxygen on the degradation
877 of bacterial membrane lipids in marine sediments, Geochim. Cosmochim. Acta, 50, 795-804, 1986.
878 Hernandez-Sanchez, M. T., Homoky, W. B., and Pancost, R. D.: Occurrence of 1-O-monoalkyl glycerol
879 ether lipids in ocean waters and sediment, Org. Geochem. 66, 1–13, 2014.
880 Hölzl, G., and Dörmann, P.: Structure and function of glycoglycerolipids in plants and bacteria, Progr.
881 Lip. Res. 46, 225–243, 2007.
882 Hurley, S. J., Elling, F. J., Könneke, M., Buchwald, C., Wankel, S. D., Santoro, A. E., Lipp, J. S., Hinrichs,
883 K.-U., and Pearson, A.: Influence of ammonia oxidation rate on thaumarchaeal lipid composition and
884 the TEX86 temperature proxy, Proc. Natl. Acad. Sci. USA, 113, 7762-7767, 2016.
885 Kalvelage, T., Lavik, G., Jensen, M. M., Revsbech, N. P., Löscher, C., Schunck, H., Desai, D. K., Hauss,
886 H., Kiko, R., Holtappels, M., LaRoche, J., Schmitz, R. A., Graco, M. I., and Kuypers, M. M. M:
887 Aerobic microbial respiration in oceanic oxygen minimum zones, PLoS ONE, 10(7):e0133526, 2015.
888 Karstensen, J., Stramma L., and Visbeck M.: Oxygen minimum zones in the eastern tropical Atlantic and
889 Pacific oceans, Progr. Oceanogr., 77, 331-350, 2008.
890 Kato, T., Yamaguchi, Y., Hirano, T., and Yokoyama, T.: Unsaturated hydroxy fatty acids, the self
891 defensive substances in rice plant against rice blast disease, Chem. Let., 409-412, 1984.

892 Keeling, R. F., Körtzinger, A., and Gruber N.: Ocean deoxygenation in a warming world, Annu. Rev.
893 Marine. Sci., 2, 199–229, 2010.
894 Kharbush, J. J., Allen, A. E., Moustafa, A., Dorrestein, P.C., Aluwihare, L. I.: Intact polar diacylglycerol
895 biomarker lipids isolated from suspended particulate organic matter accumulating in an
896 ultraoligotrophic water column, Org. Geochem., 100, 29-41, 2016.
897 Lam, P. and Kuypers, M. M. M.: Microbial nitrogen cycling processes in oxygen minimum zones, Annu.
898 Rev. Marine. Sci., 3, 317–345, 2011.
899 Landry, M. R., Selph, K. E., Taylor, A.G., Décima, M., Balch, W. M., and Bidigare R. R.: Phytoplankton
900 growth, grazing and production balances in the HNLC equatorial Pacific, Deep Sea Res. I, 58, 524-
901 535, 2011.
902 Lavín, M. F., Fiedler, P. C., Amador, J. A., Balance, L. T., Färber-Lorda, J., Mestas-Nuñez, A. M.: A
903 review of eastern tropical Pacific oceanography: Summary, Progr. Oceanogr., 69, 391-398, 2006.
904 Lee C., and Cronin C.: Particulate amino acids in the sea: Effects of primary productivity and biological
905 decomposition, J. Mar. Res., 42, 1075-1097, 1984.
906 Lehninger A. L.: Oxidation of fatty acids, in: Biochemistry, New York: Worth, 417-432, 1970.
907 Lengger, S. K., Hopmans, E. C., Sinninghe Damsté, J. S., and Schouten, S.: Comparison of extraction and
908 work up techniques for analysis of core and intact polar tetraether lipids from sedimentary
909 environments, Org. Geochem., 47, 34–40, 2012.
910 Lin, X., Wakeham, S. G., Putnam, I. F., Astor, Y. M., Scranton, M. I., Chistoserdov, A. Y., and Taylor, G.
911 T.: Comparison of vertical distributions of prokaryotic assemblages in the anoxic Cariaco Basin and
912 Black Sea by use of fluorescence in situ hybridization, Appl. Environ. Microbiol., 72, 2679-2690,

Deleted: Reichart, G.-J., Nierop, K. G. J.,

Deleted: Intact polar and core glycerol dibiphytanyl glycerol tetraether lipids in the Arabian Sea oxygen minimum zone. Part II: Selective preservation and degradation in sediments and consequences for the TEX86

Deleted: Geochim. Cosmochim. Acta

Deleted: 98

Deleted: 24

Deleted: 258

2006.

Lincoln, S. A., Wai, B., Eppley, J. M., Church, M. J., Summons, R. E. and DeLong, E. F.: Planktonic Euryarchaeota are a significant source of archaeal tetraether lipids in the ocean, Proc. Natl. Acad. Sci. USA, 111, 9858–9863, 2014.

Lynch, D. V., and Dunn, T. M.: An introduction to plant sphingolipids and a review of recent advances in understanding their metabolism and function, New Phytol., 161, 677-702, 2004.

Ma, Y., Zeng, Y., Jiao, N., Shi, Y., and Hong, N.: Vertical distribution and phylogenetic composition of bacteria in the Eastern Tropical North Pacific Ocean, Microbiol. Res., 164, 624-663, 2009.

Maas, A. E., Frazar, S. L., Outram, D.M., Seibel, B. A., and Wishner, K. F.: Fine-scale vertical distributions of macroplankton and micronekton in the Eastern Tropical North Pacific in association with an oxygen minimum zone, J Plankt. Res., 36, 1557-1575, 2014.

Martin, J. H., Knauer, G. A., Karl, D. M., and Broenkow, W. W.: VERTEX: carbon cycling in the northeast Pacific, Deep-Sea Research 34, 267-285, 1987.

Matos, A. R., and Pham-Thi, A.-T.: Lipid deacylating enzymes in plants: Old activities, new genes. Plant Physiol. and Biochem. 47, 491-503, 2009.

Meador, T. B., Gagen, E. J., Loscar, M. E., Goldhammer, T., Yoshinaga, M. Y., Wendt, J., Thomm, M., and Hinrichs, K.-U.: Thermococcus kodakarensis modulates its polar membrane lipids and elemental composition according to growth state and phosphate availability, Front. Microbiol., 5:10, doi:10.3389/fmicb.2014.00010, 2014.

Mileykovskaya, E., and Dowhan, W.: Cardiolipin membrane domains in prokaryotes and eukaryotes, Biochim. Biophys. Acta 1788, 2084–2091, 2009.

943 Morita, Y. S., Yamaryo-Botte, Y., and Miyanagi, K.: Stress-induced synthesis of phosphatidylinositol 3-
944 phosphate in mycobacteria, J. Biol. Chem. 285, 16643-16650, 2010.
945 Neal, A. C., Prahl, F. G., Eglinton, G., O'Hara, S. C. M., and Corner, E. D. S.: Lipid changes during a
946 planktonic feeding sequence involving unicellular algae, Elminius Nauplii and Adult Calanus, J. Mar.
947 Biol. Assoc. UK, 66, 1-13, 1986.
948 Neidleman, S. L.: Effects of temperature on lipid unsaturation: Biotechnology and Genetic Engineering
949 Reviews, 5:1, 245-268, 1987.
950 Oliver, J. D., and Colwell, R. R.: Extractable lipids of gram-negative marine bacteria: Phospholipid
951 composition, J. Bacteriol. 114, 897-908, 1973.
952 Olson, M. B., and Daly, K. L.: Micro-grazer biomass, composition and distribution across prey resource
953 and dissolved oxygen gradients in the far eastern tropical north Pacific Ocean, Deep Sea Res. I, 75,
954 28-38, 2014.
955 Okuyama, H., Kogame, K., and Takeda, S.: Phylogenetic significance of the limited distribution of
956 octadecapentaenoic acid in prymnesiophytes and photosynthetic dinoflagellates, Proc. NIPR Symp.
957 Polar Biol., 6, 21–26, 1993.
958 Parsons, T. R., Takahashi, M., and Hargrave B. (Eds.): Biological Oceanographic Processes, 3rd ed.,
959 Pergamon Press, NY, 1984.
960 Paulmier, A., and Ruiz-Pino, D.: Oxygen minimum zones (OMZs) in the modern ocean, Progr. Oceanogr.
961 80, 113-128, 2009.
962 Pennington, J. T., Mahoney, K. L., Kuwahara, V. S., Kolber, D. D., Clienes, R., Chavez, F. P.: Primary
963 production in the eastern tropical Pacific: A review, Progr. Oceanogr., 69, 285-317, 2006.

964 Pitcher, A., Villanueva, L., Hopmans, E. C., Schouten, S., Reichart, G.-J. and Sinninghe Damsté, J. S.:
965 Niche segregation of ammonia-oxidizing archaea and anammox bacteria in the Arabian Sea oxygen
966 minimum zone, ISME J., 5, 1896–1904, 2011.
967 Podlaska, A., Wakeham, S. G., Fanning, K. A., and Taylor, G. T.: Microbial community structure and
968 productivity in the oxygen minimum zone of the eastern tropical North Pacific, Deep-Sea Res. Part I,
969 66, 77–89, 2012.
970 Popendorf, K., Lomas, M., and Van Mooy, B.: Microbial sources of intact polar diacylglycerolipids in the
971 Western North Atlantic Ocean, Org. Geochem. 42, 803-811, 2011a.
972 Popendorf, K. J., Tanaka, T., Pujo-Pay, M., Lagaria, A., Courties, C., Conan, P., Oriol, L., Sofen, L. E.,
973 Moutin, T., and Van Mooy, B. A. S.: Gradients in intact polar diacylglycerolipids across the
974 Mediterranean Sea are related to phosphate availability, Biogeosci. 8, 3733–3745, 2011b.
975 Prahl, F. G., Eglinton, G., Corner, E. D. S., O'Hara, D. C. M., and Forsberg, T. E. V.: Changes in plant
976 lipids during passage through the gut of Calanus, J. Mar. Biol. Assoc. UK, 1984.
977 Rabinowitz, G. B.: An introduction to nonmetric multidimensional scaling, Amer. J. Polit. Sci., 343-90,
978 1975.
979 Rappé, M. S., and Giovannoni, S. J.: The uncultured microbial majority, Annu. Rev. Microbiol., 57, 369-
980 394, 2003.
981 Rojas-Jiménez, K., Sohlenkamp, C., Geiger, O., Martínez-Romero, E., Werner, D., and Vinuesa, P.: A
982 ClC chloride channel homolog and ornithine-containing membrane lipids of rhizobium tropici
983 CIAT899 are involved in symbiotic efficiency and acid tolerance, Mol. Plant-Microbe Interact., 18,
984 1175–1185, 2005.

985 Rush, D., Wakeham, S. G., Hopmans, E. C., Schouten, S., and Damsté, J. S. S.: Biomarker evidence for
986 anammox in the oxygen minimum zone of the Eastern Tropical North Pacific, Org. Geochem., 53,
987 80–87, 2012.
988 Rütters, H., Sass, H., Cypionka, H., and Rullkötter, J.: Monoalkylether phospholipids in the sulfate-
989 reducing bacteria Desulfosarcina variabilis and Desulforhabdus amnigenus, Arch. Microbiol., 176,
990 435–442, 2011.
991 Schouten, S., Pitcher, A., Hopmans, E. C., Villanueva, L., Van Bleijswijk, J., and Sinninghe Damsté, J.
992 S.: Intact polar and core glycerol dibiphytanyl glycerol tetraether lipids in the Arabian Sea oxygen
993 minimum zone: I. Selective preservation and degradation in the water column and consequences for
994 the TEX86, Geochim. Cosmochim. Acta, 98, 228–243, 2012.
995 Schubotz, F., Wakeham, S. G., Lipp, J., Fredricks, H. F., and Hinrichs, K.-U.: Detection of microbial
996 biomass by intact polar membrane lipid analysis in the water column and surface sediments of the
997 Black Sea, Environ. Microbiol., 11, 2720-2734, 2009.
998 Sebastian, M., Smith, A. F., González, J. M., Fredricks, H. F., Van Mooy, B., Koblížek, M., Brandsma,
999 J., Koster, G., Mestre, M., Mostajir, B., Pitta, P., Postle, A. D., Sánchez, P., Gasol, J. M., Scanlan, D.
1000 J., and Chen, Y.: Lipid remodelling is a widespread strategy in marine heterotrophic bacteria upon
1001 phosphorus deficiency, ISME J, 10, 968–978, 2016.
1002 Seibel, B.A.: Critical oxygen levels and metabolic suppression in oceanic oxygen minimum zones, J. Exp.
1003 Biol., 214, 326-336, 2011.
1004 Seidel, M., Graue, J., Engelen, B., Köster, J., Sass, H., and Rullkötter, J.: Advection and diffusion
1005 determine vertical distribution of microbial communities in intertidal sediments as revealed by

combined biogeochemical and molecular biological analysis, Org. Geochem., 52, 114–129, 2012.

Shanks, A. L., and Reeder, M. L.: Reducing microzones and sulfide production in marine snow. Marine Ecology Press Series 96, 43-47, 1993.

Siegenthaler P.-A.: Molecular organization of acyl lipids in photosynthetic membranes of higher plants, in: Lipids in Photosynthesis, Siegenthaler, P.-A., and Murata, N. (Eds). Dordrecht, the Netherlands: Kluwer Academic Publishers, 119–144, 1998.

Sohlenkamp, C., López-Lara, I. M., and Geiger, O.: Biosynthesis of phosphatidylcholine in bacteria, Progr. Lip. Res., 42, 115–162, 2003.

Sollai, M., Hopmans, E. C., Schouten, S., Keil, R. G., and Sinninghe Damsté, J.S.: Intact polar lipids of Thaumarchaeota and anammox bacteria as indicators of N cycling in the eastern tropical North Pacific oxygen-deficient zone, Biogeosci., 12, 4833-4864, 2015.

Stevens H., and Ulloa, O.: Bacterial diversity in the oxygen minimum zone of the eastern tropical South Pacific, Environ. Microbiol., 10, 1244–1259, 2008.

Stramma, L., Johnson, G. C., Sprintall, J., and Mohrholz, V.: Expanding Oxygen-Minimum Zones in the Tropical Oceans, Science, 320, 655-658, 2008.

Stramma, L., Schmidtko, S., Levin, L. A., and Johnson, G. C.: Ocean oxygen minima expansions and their biological impacts, Deep Sea Res. I, 57, 587-595, 2010.

Sturt, H. F., Summons, R. E., Smith, K.E., Elvert, M., Hinrichs, K.-U.: Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry - new biomarkers for biogeochemistry and microbial ecology, Rapid Comm. Mass Spec., 18, 617-628, 2004.

Taylor, G. T., Iabichella, M., Ho, T.-Y., Scranton, M. I., Thunell, R. C., Muller-Karger, F., and Varela R.: Chemoautotrophy in the redox transition zone of the Cariaco Basin: A significan midwater source of organic carbon production, Limol. Oceanogr., 46, 148-163, 2001.

Tiano, L., Garcia-Robledo, E., Dalsgaard, T., Devol, A. H., Ward, B. B., Ulloa, O., Canfield, D. E., and Revsbech, N. P.: Oxygen distribution and aerobic respiration in the north and south eastern tropical Pacific oxygen minimum zones, Deep Sea Res. I, 94, 173-183, 2014.

Turich, C., and Freeman, K. H.: Archaeal lipids record paleosalinity in hypersaline systems, Org. Geochem. 42, 1147-1157, 2011.

Ulloa, O., Canfield, D., DeLong, E. F., Letelier, R. M., and Stewart, F. J.: Microbial oceanography of anoxic oxygen minimum zones, Proc. Natl. Acad. Sci., USA 109, 15996-16003, 2012.

Valentine, R. C., and Valentine, D. L.: Omega-3 fatty acids in cellular membranes: a unified concept, Progr. Lip. Res. 43, 383–402, 2004.

Van Mooy, B. A. S., and Fredricks, H. F.: Bacterial and eukaryotic intact polar lipids in the eastern subtropical South Pacific: Water-column distribution, planktonic sources, and fatty acid composition, Geochim. Cosmochim. Acta, 74, 6499–6516, 2010.

Van Mooy, B. A. S., Fredricks, H. F., Pedler, B. E., Dyhrman, S. T., Karl, D. M., Koblížek, M., Lomas, M. W., Mincer, T. J., Moore, L. R., Moutin, T., Rappé, M. S., and Webb, E. A.: Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity, Nature, 458, 69–72, 2009.

Van Mooy, B. A. S., Rocap, G., Fredricks, H. F., Evans, C. T., and Devol, A. H.: Sulfolipids dramatically decrease phosphorus demand by picocyanobacteria in oligotrophic marine environments, Proc. Natl. Acad. Sci. USA, 103, 8607–8612, 2006.

Vardi, A., Van Mooy, B. A. S., Fredricks, H. F., Popendorf, K. J., Ossolinski, J. E., Haramty, L., and Bidle, K. D.: Viral glycosphingolipids induce lytic infection and cell death in marine phytoplankton, Science, 326, 861-865, 2009.

Wada, H., and Murata, N.: Membrane Lipids in cyano- bacteria, in: Lipids in Photosynthesis: Structure, Function and Genetics, Siegenthaler, P., and Murata, N. (Eds), Dordrecht, the Netherlands: Kluwer Academic Publishers, 65–81, 1998.

Wakeham, S. G., Turich, C., Schubotz, F., Podlaska, A., Li, X. N., Varela, R., Astor, Y., Sáenz, J. P., Rush, D., Sinninghe Damsté, J. S., Summons, R. E., Scranton, M. I., Taylor, G. T., and Hinrichs, K.-U.: Biomarkers, chemistry and microbiology show chemoautotrophy in a multilayer chemocline in the Cariaco Basin, Deep Sea Res. Part I, 63, 133–156, 2012.

Wakeham, S. G., Amann, R., Freeman, K. H., Hopmans, E. C., Jørgensen, B. B., Putnam, I. F., Schouten, S., Sinninghe Damsté, J. S., Talbot, H. M., and Woebken, D.: Microbial ecology of the stratified water column of the Black Sea as revealed by a comprehensive biomarker study, Org. Geochem., 38, 2070–2097, 2007.

Wakeham. S. G.: Monocarboxylic, dicarboxylic and hydroxy acids released by sequential treatments of suspended particles and sediments of the Black Sea, Org. Geochem. 30, 1059-1074, 1999.

Wakeham, S. G.: Reduction of stenols to stanols in particulate matter at oxic-anoxic boundaries in sea water, Nature, 342, 787-790, 1989.

Wakeham, S. G., and Canuel, E. A.: Organic geochemistry of particulate matter in the eastern tropical North Pacific Ocean: Implications for particle dynamics, J. Mar. Res., 46, 182-213, 1988.

Wakeham, S. G.: Steroid geochemistry in the oxygen minimum zone of the eastern tropical North Pacific

Ocean, Geochim. Cosmochim. Acta, 51, 3051-3069, 1987.

Williams, R. L., Wakeham, S., McKinney, R., Wishner, K. F.: Trophic ecology and vertical patterns of carbon and nitrogen stable isotopes in zooplankton from oxygen minimum zone regions, Deep Sea Res. I, 90 36-47, 2014.

Wishner, K. F., Outram, D. M., Seibel, B. A., Daly, K. L., and Williams, R. L.: Zooplankton in the eastern tropical north Pacific: Boundary effects of oxygen minimum zone expansion, Deep Sea Res. I, 79, 122-140, 2013.

Wishner, K. F., Gelfman, C., Gowing, M. M., Outram, D. M., Rapien, M., and Williams, R. L.: Vertical zonation and distributions of calanoid copepods through the lower oxycline of the Arabian Sea oxygen minimum zone, Progr. Oceanogr., 78, 163-191, 2008.

Woebken, D., Fuchs, B. M., Kuypers, M. M. M, and Aman, R.: Potential interactions of particle-associated anammox bacteria with bacterial and archaeal partners in the Namibian upwelling system, Appl. Environ. Microbiol., 73, 4648-4657, 2007.

Wörmer, L., Lipp, J. S., Schröder, J. M., and Hinrichs, K.-U.: Application of two new LC-ESI-MS methods for improved detection of intact polar lipids (IPLs) in environmental samples, Org. Geochem. 59, 10–21, 2013.

Wright, J. J., Konwar, K. M., and Hallam, S. J: Microbial ecology of expanding oxygen minimum zones, Nat. Rev. Microbiol. 10, 381-394, 2012.

Xie, S., Liu, X.-L., Schubotz, F., Wakeham, S. G., and Hinrichs K.-U.: Distribution of glcerol ether lipids in the oxygen minimum zone of the Easter Tropical North Pacific Ocean, Org. Geochem. 71, 60–71, 2014.

Yao, M., Elling, F. J., Jones, C., Nomosatryo, S., Long, C. P., Crowe, S. A., Antoniewicz, M. R., Hinrichs, K.-U., and Maresca, J. A.: Heterotrophic bacteria from an extremely phosphate-poor lake have conditionally reduced phosphorus demand and utilize diverse sources of phosphorus, Environ. Microbiol. 18, 656–667, 2015.

Zavaleta-Pastor, M., Sohlenkamp, C., Gao, J. L., Guan, Z., Zaheer, R., Finan, T. M., Raetz, C. R. H., López-Lara, I. M., and Geiger, O.: Sinorhizobium meliloti phospholipase C required for lipid remodeling during phosphorus limitation, Proc. Natl. Acad. Sci. USA, 107, 302–307, 2010.

Zhang, Y.-M., and Rock, C. O.: Membrane lipid homeostasis in bacteria, Nat. Rev. Microbiol., 6, 222–233, 2008.

Zhu, C., Wakeham, S. G., Elling, F. J., Basse, A., Mollenhauer, G., Versteegh, G. J. M., Könneke, M., and Hinrichs, K.-U.: Stratification of archaeal membrane lipids in the ocean and implications for adaptation and chemotaxonomy of planktonic archaea, Environ. Microbiol. 18, 4324-4336, 2016.

Tables

Table 1. Spearman Rank Order Correlation coefficients (r) for data combined from all four stations. Only significant correlations, where $p < 0.05$ (highly significant $p < 0.001$, in bold), are presented.

	Glycolipids						Aminolipids				Phospholipids					
	% GL	% 1G	% 2G	% SQ	GL:PL	SQ:PG	% AL	% DGTS	AL:PL	DGTS:PC	% PL	% PC	% PG	% PE	% PME	% PDME
Depth	-0.32		**-0.7**	**-0.67**	-0.41	**-0.76**										
Fluorescence			**0.63**	**0.67**		**0.65**										
POC			**0.61**	**0.6**		**0.6**										
TN			**0.66**	**0.62**		**0.63**										
Oxygen	**0.57**	0.3	**0.48**	0.35	**0.55**	**0.58**			0.36		**-0.49**		-0.38	-0.33	-0.46	-0.52
Temperature	0.3		**0.52**	**0.63**	0.39	**0.69**										
Chl a	0.35		**0.72**	**0.71**	0.42	0.78										-0.33
Phosphate			**-0.62**	**-0.53**	-0.4	**-0.56**										0.36
Nitrate			**-0.53**	**-0.49**		-0.38										
Nitrite		-0.33													0.3	
Ammonium							0.41	0.42	0.35	0.4						
N:P			-0.3	-0.32									-0.36			

Abbreviations: GL – glycolipids, 1G – monoglycosyl, 2G – diglycosyl, SQ – sulfoquinovosyl, PL – phospholipids, AL – aminolipids, DGTS – diacylglyceryl trimethyl homoserine, PC – phosphatidyl choline, PG – phosphatidyl glycerol, PE – phosphatidyl ethanolamine, PME – phosphatidyl methyl-ethanolamine, PDME – phosphatidyl dimethyl-ethanolamine

Figures

Figure 1. a) Map of ETNP with R/V *Seward Johnson* (November 2007) cruise sampling stations investigated in this study.

Figure 2. Depth profiles of (a) oxygen and temperature, (b) chlorophyll-α and transmissivity, (c) particulate organic matter (POC) and C:N, (d) intact polar lipid (IPL) to POC ratio and IPL concentration, and (e) absolute cell abundance and relative proportions of archaeal cells (data from Podlaska et al. (2012)). C:N (SPM) is total carbon over total nitrogen of the solid phase collected by water filtration. Note that C:N, POC and IPL/POC are only analyzed for <53 μm particle fraction. Also depicted are the different geochemical zones in the water column.

Figure 3. Depth profiles of (a) nitrate, nitrite, and ammonium, (b) phosphate and N:P, (c) total non-archaeal (non-isoprenoidal) phospholipids, glycolipids and (d) aminolipids shown as percent of total intact polar lipids and ratios of non-phospholipids to phospholipids for DGTS to PC-DAG (e) SQ-DAG to PG-DAG, (e), and 1G-DAG to PE-DAG. Also depicted are the different geochemical zones in the water column.

Figure 4. Relative abundance of (a) major and (b) minor IPLs at sampled depths of stations 1, 2, 5, and 8 in the ETNP. Major IPLs are defined as those comprising more than 10% of total IPLs (minor compounds comprised less than 10%) at more than one depth horizon at the four stations. Also depicted are the different geochemical zones in the water column.

Figure 5. Changes in average carbon atoms (CA) and number of double bond equivalents (DB) of the alkyl side chains of major IPLs detected at stations 1, 2, 5 and 8 in the ETNP.

Figure 6. Nonmetric multidimensional scaling (NMDS) ordination plot assessing the relationship between IPL biomarkers, sampling depths and geochemical parameters in the ETNP (stress=0.125). Squares represent the water depth of each sample and are color-coded according to the defined geochemical zonation. Filled circles stand for lipid distribution of major IPLs and open circles for minor IPLs on the ordination. Vector lines of geochemical parameters are weighted by their p-values with each NMDS axis.