Biomarkers, chemistry and microbiology show chemoautotrophy in a multilayer chemocline in the Cariaco Basin

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Abstract
The Cariaco Basin is the world’s largest truly marine anoxic basin. We have conducted a comprehensive multidisciplinary investigation of the water column (42–750 m) bracketing the redox boundary (a 250-m thick “chemocline”) of the Cariaco Basin to evaluate linkages between lipid biomarkers, distributions of major dissolved chemical species, and the microbial community and associated redox processes. Our multidimensional data set includes: hydrography, water column chemistry, microbial distributions and rates, and lipid biomarkers. Multivariate statistical analysis of this data set partitions the investigated water column into 5 distinct zones, each characterized by different chemistries, microbiologies and biomarker compositions. The core of this chemocline is a 25-m thick suboxic zone where both dissolved oxygen and sulfide were below detection limits, bacterial and archaeal cell numbers and the rate of chemoautotrophic (dark) carbon fixation are elevated, and dissolved chemical species and bacterial and archaeal lipid biomarkers are indicative of tightly coupled cycles of carbon, nitrogen, and sulfur through chemoautotrophy.

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1. Introduction
Permanently anoxic marine basins such as the Cariaco Basin are ideal natural laboratories for investigating relationships between physical controls on nutrient dynamics and primary production, biogenic particle export and transformations under oxic, suboxic and anoxic conditions, and preservation of paleoceanographic records in underlying sediments. The US-Venezuelan CARIACO Ocean Time Series Program (Carbon Retention in a multilayer chemocline in the Cariaco Basin) has collected more than 15 years of monthly measurements of physical, chemical and biological properties in the Cariaco Basin, complemented by SeaWIFS satellite observations and biannual process-study cruises (Thunell et al., 2001, 2007; Muller-Karger et al., 2004; Göth et al., 2009 for background). High seasonal coastal surface productivity and restricted water exchange between the Caribbean Sea and the deep Cariaco Basin maintain a sulfidic (“euxinic”) zone from ~260 m to the seafloor at ~1400 m (Astor et al., 2003; Scranton et al., 2006). Between oxygenated surface waters and sulfidic bottom waters lies a well-developed ~250-m thick zone of strong chemical gradients and associated microbial-biological community, hereafter termed “the chemocline”, in which lipid biomarker distributions are markedly different.

Biogeochemical redox processes within the chemocline support a diverse microbial community (Madrid et al., 2001; Lin et al., 2006, 2007, 2008; Stocke et al., 2003; Taylor et al., 2006) that produces diagnostic lipid biomarkers (Wakeham, 1990; Freeman et al., 1994; Wakeham et al., 2004). Various oxidants (O2, MnO2, Fe2O3, NO3-, S2O32-, SO42-, S0) and reductants (H2S, NH4+, CH4, Fe2+, Mn2+) could
support chemoautotrophy (Scranton et al., 2001; Scranton, 1988; Taylor et al., 2001; Ho et al., 2002, 2004; Hayes et al., 2006; Percy et al., 2008; Li et al., 2008, 2012). Denitrifying, metal-reducing, and ammonium and sulfide oxidizing microbial populations are all active within different depth intervals. Chemoautotrophy in the Cariaco Basin equates to 10–130% of contemporaneous primary production and responds to interannual and decadal changes in surface productivity and deep-water ventilation rather than short-term surface processes of seasonal upwelling and blooms (Taylor et al., 2001). As a result, the flux of organic carbon (OC) captured in sediment traps below the chemocline frequently exceeds the flux exported from above, further implicating mid-water chemoautotrophic production as an important secondary source of OC in the water column and to the sediments in addition to surface water photoautotrophy (Taylor et al., 2001). Anoxic and laminated sediments of the Cariaco Basin preserve a record of Holocene climate change, including changes in upwelling intensity, planktonic community structure and regional rainfall resulting from the shifting position of the Inter-Tropical Convergence Zone (ITCZ) (Goff et al., 2009, and references cited). Recognizing the significance of mid-water chemoautotrophy from a lipid biomarker perspective aids in identifying water column sources of biomarkers as proxies for biological processes and climate change, especially for past oceans characterized by euxinic conditions and extensive organic carbon deposition. The underlying causes of oceanic anoxic events (OAEs) that may lead to organic-rich sediments deposited under oxygen-deficient water column conditions are the subject of ongoing investigations, given recent suggestions that ocean warming and increased stratification caused by global climate change lead to declining dissolved oxygen in the interior of the ocean (Keeling et al., 2010).

This study uses a high resolution sampling scheme to generate a multidimensional data set to help define linkages between lipid biomarkers, distributions of major dissolved chemical species, and the microbial community. Our data set includes: hydrography [temperature, fluorescence, transmissivity and dissolved oxygen], chemistry [particulate organic carbon (POC), total particulate nitrogen (TN), δ13CPOC, CHA, nutrients (NO₃⁻, NO₂⁻, NH₄⁺), sulfur species (H₂S, S₂O₃²⁻, SO₃²⁻, SO₄²⁻)], microbial distributions and rates [bacterial and archaeal cell numbers, abundances of β-, and ε-proteobacteria, sulfate-reducing and anammox bacteria; heterotrophic and chemoautotrophic production rates] and lipid biomarkers [intact polar lipids, bacteriohopanepolysols, ladderane lipids, fatty acids and free apolar compounds]. Our results show that a multilayered chemocline spans roughly 250 m of the water column, from the oxycline into the anoxic zone. Within this greater chemocline, a 25-m suboxic zone and the transition into anoxic waters together harbor a complex assemblage of chemoautotrophic microorganisms that connect the cycles of carbon, nitrogen and sulfur and act as a “hotspot” of production of new organic matter (OM).

2. Methods

2.1. Site description and sampling

The Cariaco Basin is located on the continental shelf of northern Venezuela (Fig. 1). A shallow sill (< 150 m) isolates it from the Caribbean Sea, and a second, deeper saddle (~1000 m) restricts circulation between the two deep 1400-m sub-basins. Upwelling, regional rainfall, primary productivity and particle export are all driven by the seasonal migration of the ITCZ. In winter, the ITCZ is below the equator promoting sustained easterly Trade Winds, strong upwelling and export of autochthonous OM. During summer the ITCZ is farther north, bringing high precipitation and increased sedimentation of allochthonous lithogenic material from the Venezuelan uplands (Muller-Karger et al.,

![Fig. 1. Map showing the location of the Cariaco Basin, and the effect of the ITCZ position on seasonal upwelling (boreal winter) during November sampling, and high precipitation and run-off (summer). Suspended particles were collected in November 2007 (CAR-139) at the CARIACO time-series site in the Eastern Basin.](image)
2004; Goñi et al., 2009). The lithogenic and OM records preserved in Cariaco sediments suggest significant variation in the location and strength of the ITCZ over regional climate cycles. All samples were collected during the CAR-139 cruise in late November, 2007, at the CARIACO time-series site (10°30'N, 64°40'W; Fig. 1). Suspended particulate matter (SPM) for biomarker analyses was collected using two McLane WTS-LV in situ filtration systems deployed in tandem on 21–22 November using B/O Paraguaucha operated by Estación de Investigaciones Marinas de Margarita (EDIMAR). Ten SPM samples were collected between nominal depths of 205–346 m (205, 220, 236, 245, 256, 270, 276, 296, 324, 346), along with a sample at the fluorescence maximum (42 m). On November 28 (bad weather forced the Paraguaucha back to port; 28 November was the previously scheduled sediment trap turn-around cruise), a deep anoxic zone sample was collected at 750 m using EDIMAR’s B/O Hermano Gines. A Seabird CTD on the hydrowire directly beneath the pumps provided real-time depth (used to estimate the depths of the pumps), temperature, salinity, fluorescence (WetLab ECO-AFL), beam attenuation (WetLab C-star transmissometer) and dissolved oxygen (SBE 43 oxygen probe). Between 200–850 liters were filtered during ~2–h pump deployments. Two particle sizes were collected using a 53 μm Nitex prefilter and two stacked 142 mm Whatman GF/F filters (nominally 0.7 μm; ashed at 500 °C for 8 h). Only the GF/Fs, representing 0.7–53 μm material, were analyzed in this investigation. Filters were frozen immediately and returned to the laboratory frozen. Logistical constraints required that chemical and microbial samples be collected on a separate leg of CAR-139 on 30 November. We assume that the chemocline is relatively stable and that the temporal spread in sampling has minimal impact on comparing the multiple parameters we have measured, but in reality this might be an oversimplification. In addition, we are comparing microbiological distributions obtained using 0.22 μm membrane filters with biomarker results obtained using 0.7 μm GF/F filters. Because GF/F may undersample the microbial community (Lee et al., 1995; Gasol and Moran, 1999) we stacked two GF/F together to try to minimize any discrimination while still filtering the large volumes needed for biomarker analyses.

2.2. Elemental analysis

Particulate organic carbon (POC), total nitrogen (TN) and stable carbon isotope composition of bulk OM were determined on 14 mm-diameter subsamples of each filter. The plucks were acidified in HCl vapor in a dessicator for 12 h to remove inorganic carbon and analyzed with a ThermoFinnigan Flash EA Series 1112 interfaced to a ThermoFinnigan Delta V isotope ratio mass spectrometer. Sample stable isotopes, % C and % N values were calibrated against internal laboratory chitin powder standards which in turn had previously been cross-calibrated against USGS 40 and 41 international isotope standards (Qi et al., 2004).

2.3. Lipid analysis

Lipids were Soxhlet-extracted using dichloromethane:methanol (DCM:MeOH; 9:1 v/v) for 8 h. Extracted lipids were partitioned into DCM against 5% NaCl solution and dried over Na2SO4. High performance liquid chromatography–mass spectrometry (HPLC–MS) was used to investigate intact polar lipids (IPL: glycolipids (GL), phospholipids (PL) and intact glyco-glycerol dialkyl glycerol tetraethers (IP-GDGT)) on one split of the lipid extracts. Bacteriohopanepolysols (BHP), core ladderane lipids, and core GDGT were determined by HPLC–MS on separate splits of lipid extracts. Apolar lipids and fatty acids were measured on saponified splits of extracts by gas chromatography–mass spectrometry (GC–MS).

Our choice of Soxhlet extraction is a compromise, but it was used in our previous IPL work in the Black Sea (Schubotz et al., 2009). Selection of extraction procedure depends on both the target lipids and the sample matrix. For example, the phosphate buffered Bligh–Dyer method is often considered superior for living biomass (Bligh and Dyer, 1959; Lipp et al., 2008), but Soxhlet extraction has traditionally been used for sediments and soils. Soxhlet extraction is frequently replaced by accelerated solvent extraction (ASE). However, both Soxhlet extraction and ASE lead to poor recoveries of intact ladderane lipids. A comparison of extraction techniques for archaeal lipids showed that extraction efficiencies differed significantly depending on whether core GDGT or IP-GDGT were targeted, and on whether cells or environmental samples (particulate matter and sediments) were being extracted (Huguet et al., 2010).

2.3.1. Intact polar lipids (IPL)

IPL were measured on a ThermoFinnigan Surveyor LC system coupled to a ThermoFinnigan LCQ Deca XP Plus ion-trap mass spectrometer via electrospray interface (HPLC–ESI–IT–MS), under positive and negative ionization modes with automated data-dependent fragmentation of base peak ions up to MS3 (Schubotz et al., 2009). Authentic standards included: diacylglycerol phosphatidylethanolamine (PE-DAG), diacylglycerol phosphoryl-(N,N')-dimethylethanolamine (PDME-DAG), diacylglycerol phosphatidylglycerol (PG-DAG), diacylglycerol phosphatidylcholine (PC-DAG), monoglycosyldiacylglycerol (Gly-DAG), diglycosyldiacylglycerol (2Gly-DAG), and glycosidic phosphatidylglycerolbiphenylalanitreaether (Gly-GDGT-PG; Avanti Polar Lipids, USA). Sulfoquinovosyldiacylglycerol (SQ-DAG), betaine lipids (BL), ornithine lipids (OL), and glycosylceramides (Gly-Cer) were tentatively identified using published mass spectra (Kim et al., 1997; Geiger et al., 1999; Schubotz et al., 2009). We did not distinguish between the two isomers of betaine lipids (BL), diglycosyceril trimethylhomoserine DGTS and diacylglycericyl-hydroxymethyl-trimethyl-β-alanine DGTA due to the lack of standards. PE- and PME-diether glycerol (DEG) and -acetyl ether glycerol (AEG) core structures were not distinguished due to co-elution. Core lipid composition of the IPL, e.g. distribution of acyl moieties, was determined in both positive and negative ionization modes (Sturt et al., 2004; Schubotz et al., 2009). Quantification of IPL was achieved using known amounts of an injection standard: 1-O-hexadecyl-2-acetoyl-sn-glycero-3-phosphocholine (PAF; Avanti Polar Lipids, USA). External calibration curves were compiled for PE-DAG, PG-DAG, PC-DAG and Gly-GDGT-PG. Response factors relative to PAF varied between 1.2 and 2.1. In cases for which response factors were not determined based on authentic standards, we assumed a mean response factor of the analyzed IPL standards, although varying degrees of unsaturation affect response. Detection limits of IPL analyses ranged between 0.003 and 0.7 ng L−1 depending on IPL class.

Chromatographic separation and identification of intact glycosyl-GDGT (Gly-GDGT) and diglycosyl-GDGT (2Gly-GDGT) with GDGT-0 to GDGT-3 and crenarchaeol as core structures was achieved using HPLC–ESI–MS of unhydrolyzed lipid extracts. Ring distributions of core GDGT were determined on aliquots of unhydrolyzed lipid extracts by HPLC–positive ion atmospheric pressure chemical ionization mass spectrometry (HPLC–APCI–MS) using a ThermoFinnigan Surveyor HPLC system coupled to a ThermoFinnigan LCQ Deca XP Plus ion trap mass spectrometer (Hopmans et al., 2000; Lipp and Hinrichs, 2009). Relative ring distributions were determined from mass chromatograms of the M+ ions.

2.3.2. Bacteriohopanepolysols (BHP)

BHP (as acetates) were analyzed by HPLC–APCI–MS using a ThermoFinnigan Surveyor LC coupled to a ThermoFinnigan LTQ–MS...
in APCI-positive ion mode (Talbot et al., 2003a, b, 2007a, b). LTQ–MS conditions were optimized using an extract of *Rhodopseudomonas palustris* and tuning to the molecular ion of acetylated bacteriohopanepetetrol (BHT, *m/z* 655). Structural identification was based on retention time and characteristic fragmentation patterns determined from extracts of *R. palustris* and *Crocophaga watsonii* or reported previously (Talbot et al., 2003c, 2008; Talbot and Farrimond, 2007). Quantitation used an external calibration curve for BHT isolated from *R. palustris* (Saenz et al., 2011). 3z,12z-Dihydroxy-5β-pregn-20-one, 3,12-diacetate (PD) was chosen as an internal standard because of its structural similarity to hopanoids and because it has a retention time that does not overlap with the range of BHT retention times. Both BHT and PD exhibited linearity in response factor over 4 orders of magnitude. Concentrations for individual BHPs were calculated relative to the peak area of the PD internal standard based on the difference in response factor between BHT and PD.

### 2.3.3. Ladderane fatty acids

Splits of extracts were saponified and cleaned up over a silica nitrate column for analysis of core ladderane fatty acids (analyzed as C15 [5]-, C17 [5]-, C20 [5]- and C23 [3]-ladderane methyl esters) by HPLC–APCI–MS/MS with a ThermoScientific Quantum TSQ Ultra EM triple quadrupole mass spectrometer (Hopmans et al., 2006; modified by Rattray et al., 2008). Detection was achieved by selective reaction ion monitoring of fragments specific to each of the four core ladderane fatty acids.

### 2.3.4. Additional biomarkers

Fatty acids (FA) were determined on saponified total lipid extracts to compare with FA distributions inferred from LC–mass spectra of IPL and for their 13C isotopic compositions. FAs were methylated with CH3N2 and fatty acid methyl esters (FAME) were analyzed by gas chromatography–isotope ratio monitoring–mass spectrometry (GC–MS) with an Agilent 6890 gas chromatograph coupled to an Agilent 5979 mass spectrometer. Electron ionization (EI) and methane–chemical ionization (CI) GC–MS experiments were conducted. Concentrations of FAME were calculated using GC–MS response relative to a methylnonadecanoate (C19) injection standard. The δ13C of FAME were determined in triplicate by gas chromatography–isotope ratio monitoring–mass spectrometry using a ThermoFinnigan Trace Ultra GC coupled via a ThermoScientific Finnigan GC Combustion III to a ThermoFinnigan Delta V isotope ratio mass spectrometer. Complications associated with diazomethane derivatization of fatty acids for stable carbon isotope analysis compared to BF3-MeOH (e.g., Riley, 1994) were addressed using a C21 FA standard that was methylated at the same time as the entire set of samples and with the same fresh diazomethane. Isotopic values of FA were calculated by mass balance correction for carbon added during methylation.

Diploptene (17z-CH2-17z)-HO-hop-22-(29)-ene), pentamethylocis-sane (PMI, 2,6,19,15,19-pentamethylcicosane), squalene (2,6,10,15, 19,23-hexamethylandocta-2,6,10,14,19,23-hexaene), lycopane (2,6,10,14,19,23,27,31-octamethyltrioctane) and tetrahymanol (γ-gammaceran-3β-ol) in the non-saponifiable lipid fractions were measured by GC–MS (Wakeham et al., 2007). S(1/2)-H-Cholestane was used as a standard for quantitation.

### 2.4. Seawater chemistry

Seawater samples for water column chemistry were collected with Teflon-lined Niskin bottles on a rosette. Niskin bottles were pressurized with nitrogen and subsamples for nutrients were filtered through 0.7 μm GF/Fs. Nitrate, nitrite and ammonium were analyzed as described by Scranton et al. (2001). Sulfide, thiosulfate, sulfite and particulate elemental sulfur were analyzed following Li et al. (2008). Methane was measured in headspace by gas chromatography (Scranton et al., 2001).

### 2.5. Microbial abundances and activity

Samples for total microbial abundances were collected in 250-ml polyethylene bottles containing filtered, borate-buffered formaldehye (2% final conc.), and refrigerated. Total microbial abundances were determined by acidine orange staining and epifluorescent microscopic enumeration (Taylor et al., 2001). Duplicate water samples (90 mL) from each depth were preserved with borate-buffered formaldehye (2% final conc.) and frozen at sea. Upon return to shore, thawed samples were filtered onto 47 mm 0.2 μm Millipore GFTP membranes and frozen until hybridization (Lin et al., 2006). Abundances of specific prokaryotic clades were determined by fluorescent in situ hybridization (FISH) following protocols of Pernthaler et al. (2002). Individual wedges cut from filters were hybridized against one of the following Cy3-monolabeled rRNA oligonucleotide probes: CREN537 (*Crenarchaeota*), EURY806 (*Euryarchaeota*), EUB338 (*Bacteria*), BET42a and BONE23A = BETmix (*β*-proteobacteria), EPS682 (*α*-proteobacteria), ALF968 (*γ*-proteobacteria), GAM42a (*γ*-proteobacteria), SRB385 (sulfate-reducing bacteria) (all in Lin et al., 2006) and AMX368 (*Anaerobic ammonium-oxidizing bacteria; Schmid et al., 2003). Relative abundances of the targeted taxa were calculated as percentage of total DAPI-positive (4′,6-diamidino-2-phenylindole) inventories using epifluorescence microscopy and converted to cell concentrations. Heterotrophic bacterial net production and chemautotrophic production were measured by assimilation of 3H-leucine into protein and dark 14C-bicarbonate into particles, respectively (details in Taylor et al., 2001; Li et al., 2012). Water samples in glass-stoppered bottles were immediately spiked with 3H-leucine or 14C-bicarbonate and incubated in coolers at ambient temperatures (~25 °C for shallow samples; ~18 °C for chemocline and sulfidic samples) for up to 20 h. Particles were collected on 0.22 μm cellulose membrane filters, washed with filtered seawater, and radioassayed.

### 2.6. Statistical analysis

Statistical analyses were performed in R (http://cran.r-project.org/; Maechler et al., 2005). R is a freeware environment created and maintained by a community of users, especially for ecological analysis. We used non-metric multidimensional scaling (NMDS), a robust unconstrained non-parametric ordination method that plots similar objects close together in ordination space, typically on 1–3 axes, providing a good method for overlaying environmental data (Minchin, 1987; Legendre and Legendre, 1998). Stress < 10 is considered to yield reliable results (McCune and Grace, 2002). We used metaMDS in R (vegan package), based on Kruskal’s MDS (Oksanen et al., 2005), with the same transformation and distance measure as the cluster analysis (standardized by row totals, Bray–Curtis distance measure). The resulting ordination plot displays sampling depths and lips. The function envfit (vegan package) was used to overlay environmental factors and determine the relationship between the ordination of lipid distributions, sample depths, and environmental variables such as geochemistry and microbial distribution.

### 3. Results

#### 3.1. Water column conditions during CAR-139

Hydrographic, chemical, and microbiological data for CAR-139 (Figs. 2–4, respectively) provide multiple lines of evidence for a concentration of microbes involved in redox processes within the
Cariaco Basin chemocline. Stratification and maintenance of anoxia in this system are due to the temperature gradient (Fig. 2a), in contrast to the Black Sea where the salinity contrast controls the density structure (Scranton et al., 2001; Astor et al., 2003). Strong seasonal upwelling, reaching a maximum between February and May, may affect waters shallower than ~120 m (Scranton et al., 2006). Primary production (integrated from 0 to 100 m) at the CARIACO site ranges from about 0.45 to 6.9 g C m⁻² d⁻¹, with high production rates during spring upwelling and low during fall stratification when we sampled; OM export from the euphotic zone followed the same temporal pattern (Muller-Karger et al., 2004; Woodworth et al., 2004; E. Montes and F. Muller-Karger, personal communication; CARIACO data http://ocb.whoi.edu/jg/dir/OCB/CARIACO/). Primary production was not measured on CAR-139 (November, 2007) but was 1.1 and 0.8 g C m⁻² d⁻¹ for CAR-138 (7 October 2007) and CAR-140 (11 December 2007), respectively. During the CAR-139 sampling campaign, the fluorescence maximum was located at ~40 m (Fig. 2b). A second "lens" of light scattering particles at 240–250 m was seen as a peak in beam attenuation (Fig. 2b) and became the target depth for SPM sampling since we surmised this to be a microbe-enriched suboxic layer.

3.1.1. Water column chemistry

The chemocline is characterized by opposing gradients of oxygen, nitrate, and sulfide (Fig. 3). Remineralization of exported particulate organic matter from the euphotic zone consumes dissolved oxygen so that below ~230 m dissolved O₂ was below its detection limit of 2–5 μM (Fig. 3a). Nitrate released by OM remineralization had a broad concentration peak centered at ~150 m (~13 μM, Fig. 3b), then decreased, but showed a small secondary peak at 260 m (4.4 μM). Nitrite had a weak peak at 150 m (0.05 μM) but a stronger peak at 260 m (0.6 μM). Sulfide was first detected (~1 μM) at 260 m (Fig. 3a). Methane (Fig. 3a) and ammonium (Fig. 3b) were first detected at, respectively, 240 and 250 m, both increasing in concentration into the euxinic deeper waters.

Intermediate oxidation states of sulfur are important substrates for microbial metabolism at redox boundaries and are likely key components in chemosynthesis in the Cariaco Basin (Taylor et al., 2001; Hayes et al., 2006; Percy et al., 2008; Li et al., 2008, 2012). For CAR-139, depth profiles of thiosulfate (S₂O₃⁻), sulfite (SO₃⁻), particulate elemental sulfur (S⁰) are shown in Fig. 3c. S⁰ showed a strong concentration maximum (1.4 μM) at 260 m depth, whereas there were slightly increasing but considerably lower concentrations of S₂O₃⁻ and SO₃⁻ (0.3 and 0.1 μM, respectively) below about 250 m, with their concentrations starting to rise at about 230 m.

3.1.2. Microbial composition and activity

Microbial community structure and activity for CAR-139 (Fig. 4) were determined on the basis of cell counts and ³H and ¹³C uptake experiments. Microbiological results for other CARIACO investigations are in Taylor et al. (2001), Madrid et al. (2001) and Lin et al. (2006, 2007, 2008). Bacteria dominated the prokaryotes, being ~25-fold more abundant than Archaea (Fig. 4a). Cell numbers of both domains decreased from surface waters but exhibited secondary peaks between 200 and 300 m. Among Bacteria, high cell numbers of ε-proteobacteria (40% of DAPI-positive cells) and β-proteobacteria (11%) were measured in the core and deep chemoclines (Fig. 4b). α-Proteobacteria were ~8% of DAPI-positive cells at depths shallower than ~100 m but declined across the chemocline to ~1% in the anoxic zone (not presented in Fig. 4), γ-Proteobacteria were rarely more than ~2% of DAPI-positive cells within the suboxic zone (not presented). Anaerobic ammonium oxidizing (anammox) bacteria (Planctomycetes) were found in a very thin layer between 230 and 240 m (AMX Fig. 4c), constituting ~3% of total prokaryotes. Sulfate-reducing bacteria (SRB), accounted for 6–9% of DAPI-positive cells below ~335 m (Fig. 4c). Cell numbers for archaea are the sum of values obtained using probes [Cren537+EURY806], Cells positive with EURY806 were 2–3 fold more abundant than cells positive for Cren537 in surface waters and at 250–260 m, but Cren537-positive cells were 2–3 fold more abundant than EURY806-positive cells between 150 and 240 m.

Chemosynthetotrophic (dark carbon fixation) production during CAR-139 peaked at ~1 μM C d⁻¹ in the depth interval between 250 and 300 m compared to ~0.02 μM C d⁻¹ for carbon production via heterotrophy (Fig. 4d). Integrated chemosynthetotrophy and heterotrophy between 250 and 450 m were 53 and 1.4 mmol C m⁻² d⁻¹, respectively. For comparison, primary production rates (0–100 m)
measured before and after CAR-139 were 90 mmol C m$^{-2}$ d$^{-1}$ for CAR-138 and 67 mmol C m$^{-2}$ d$^{-1}$ for CAR-140.

3.1.3. Elemental composition and $\delta^{13}$C of SPM

POC concentration (Fig. 5 a) was highest in the euphotic zone (30 $\mu$g L$^{-1}$), and decreased to $< 10 $ $\mu$g L$^{-1}$ through the upper chemocline, increased sharply to 17 $\mu$g L$^{-1}$ at 245 m, and decreased again below that depth. The C/N (a) ratios at 245–270 m were 5.2, compared to shallower (5.8) and deeper (5.4) samples (Fig. 5 b), indicating the POM at depth to be enriched in nitrogen, possibly due to bacterial biomass rich in N compared to plankton and detritus (Goldman et al., 1987). The $\delta^{13}$CPOC of SPM decreased to $-27.6\%$ at 245–270 m relative to the values at 42 m ($-25.8\%$), and in the anoxic zone ($-23.8\%$) (Fig. 5c). For comparison, Muller-Karger (personal communication) measured a narrow range between $-22.4\%$ and $-21.3\%$ for $\delta^{13}$CPOC in sinking sediment trap material between 50 and 410 m during CAR-139, similar to the range for long-term CARIACO measurements of trap material throughout the water column (Woodworth et al., 2004). Typically, trap POC during the high productivity/high export flux spring upwelling period is 3–5% enriched in $^{13}$C compared to the lower productivity/lower flux fall stratification period we sampled. Intermediate-depth nepheloid layers occasionally advect off the continental shelf at ~100 m depth (Lorenzoni et al., 2009), but hydrographic conditions during CAR-139 were not conducive for this to occur.

3.2. Intact polar lipids (IPL)

Analysis of IPL is assumed to target viable organisms because polar phosphoglycerol or glycosidic head groups are rapidly hydrolyzed upon cell death, yielding the more stable core lipid. This assumed lability has, however, been demonstrated infrequently, and then only in sediments (White et al., 1979; Harvey et al., 1986) or via a diagenetic model (Schouten et al., 2010). In this study we make the critical assumption that the IPL we measured in the water column of the Cariaco Basin represent live cells. Concentrations are estimates due to above-mentioned methodological considerations (extraction and response factors).

The HPLC–MS technique used allows simultaneous determination in unfractionated lipid extracts of 12 major classes of IPL comprised of either phosphate-, sugar-, amino- or sulfur-based polar head groups and various hydrophobic side chains, typically fatty acids for bacteria and eukarya, and ether-bound isoprenoidal lipids for archaea. IPL include: three classes of glycolipids (GL), mono-glycosyldiaclylglycerols (GL-DAG), diglycosyldiaclylglycerols (2GL-DAG II), and sulfoquinovosyldiaclylglycerols (SQ-DAG III); six classes of phospholipids (PL), phosphatidylglycerol (PG-DAG V), diphosphatidylglycerol (DAG-DAG VI), phosphatidylethanolamine (PE-DAG VIII), and its methyl (diacylglycerol phosphatidyl-(N)-methylethanolamine PME-DAG IX) and dimethyl (diacylglycerol phosphatidyl-(N,N)-dimethylethanolamine PDME-DAG X) derivatives; betaine lipids (BL XIV and XV); ornithine lipids (OL XVI); glycerolipids (Gly-Cer IV); and intact polar glyceryl dialkyl glycerol tetraethers (IP-GDGT XVII–XIX). Total IPL concentrations (sums of the above classes) varied from 2.8 $\mu$g L$^{-1}$ at the fluorescence maximum (Fig. 6a), dropped to 0.02 $\mu$g L$^{-1}$ at 205 m, exhibited a secondary peak at 2.2 $\mu$g L$^{-1}$ at 256 m, and then declined to 0.2 $\mu$g L$^{-1}$ at 750 m. Assuming IPL to be about 85% C, they constituted 9% of POC at 42 m, 6% at 245 m, 13% at...
3.2.2. Phospholipids (PL)

Six classes of phosphoglycerides with diacyl core lipids but different polar head groups [e.g., a second glycerol moiety PG and DPG or one nitrogenous base PC, PDME, PME and PE] were present in Cariaco Basin SPM. The most abundant phospholipids were PE-DAG (VIII), PME-DAG (IX) and PDME-DAG (X), together accounting for up to ~90% of IPL, especially between 245 and 326 m. Minor amounts of diether-based phosphatidylethanolamines (PE/PME-DEG XI and XII) were also detected, <2% of IPL in and below the suboxic zone, reaching 7% of IPL at 750 m. PE-DAG, PME-DAG and PDME-DAG diacyl chain lengths ranged between 28 and 36 with core acyl groups of saturated and monounsaturated even-carbon chains, e.g., C\textsubscript{16:0}/C\textsubscript{16:0}, and some C\textsubscript{15} and C\textsubscript{17} acids, e.g., C\textsubscript{15:0}/C\textsubscript{15:0}, C\textsubscript{15:0}/C\textsubscript{16:0} (Table 1). The second most abundant class of phospholipids was PG-DAG (V) which represented significant proportions of IPL only in the oxycline (8% of IPL at 220 m) and suboxic zone (5% of IPL at 245 m). Diglyceride moieties of PG-DAG were mainly 32–36 carbon chain lengths in combinations of C\textsubscript{16:0}, C\textsubscript{16:1}, C\textsubscript{18:0} and C\textsubscript{18:1} acyl moieties. PC-DAG (VII) were generally less abundant among PL, constituting 6% of IPL at 220 m, 2% at 256 m, but 11% at 750 m. PC-DAG covered a wide range of diacyl carbon numbers (28–38). At 42 m, PC-DAG contained polyunsaturated acids (C\textsubscript{18:4}, C\textsubscript{18:5}, C\textsubscript{20:5} and C\textsubscript{20:6}) in combination with C\textsubscript{14:0}, C\textsubscript{16:0}, C\textsubscript{16:1}, and C\textsubscript{18:1}; PC-DAG below 42 m contained much lower abundances of PUFAs. Below 256 m diphosphatidylglycerols (DPG-DAG VI) and lyso-DPG-DAG (XIII) (1–4% at 270–346 m) were detected in minor amounts, with combinations of C\textsubscript{16:0}, C\textsubscript{16:1}, C\textsubscript{17:1}, C\textsubscript{17:0} and C\textsubscript{18:0} acids. The average PE/PME/PDME-DAG in the euphotic zone contained 33 carbon atoms but only one double bond equivalent (Fig. 8d); carbon numbers decreased slightly with depth but the degree of unsaturation remained low. PG-DAG did not vary much with depth, containing generally 33.5 carbon atoms and 1.5 double bond equivalents (data not shown). PC-DAG ranged from on average 34 carbon atoms and three double bond equivalents in the euphotic zone to ~32 carbon atoms and one double bond in the anoxic zone (Fig. 8c).

3.2.3. Betaine lipids (BL)

Betaine lipids (XIV and XV) are amino acid-containing diacylglyceryl lipids. They represented 17% of IPL at 42 (Fig. 7), dominated the upper chemocline (90% of IPL), decreased to lower levels in the core and deep chemocline zones (3–4% of IPL), but were enriched again in the deep anoxic zone (20% of IPL). In the euphotic and upper chemocline zones, the major acyl groups possessed 30–40 carbon atoms, with even chained acyl moieties C\textsubscript{14:0}, C\textsubscript{16:0}, C\textsubscript{16:1}, C\textsubscript{18:0}, C\textsubscript{18:1} and C\textsubscript{22:0} (ave ~34 carbon atoms invariant with depth, and ~2 double bond equivalents at 42 m decreasing to ~1 double bond equivalent in the chemocline; Fig. 8b). Even carbon acyl chain lengths are hereafter termed “even-chain BL”. In contrast, BL containing significant amounts of odd-carbon numbered diacyl chains (e.g., C\textsubscript{31:0}, C\textsubscript{31:1}, C\textsubscript{33:0}, C\textsubscript{33:1}; “odd-chain BL”) consisting of odd-carbon C\textsubscript{15:0}, C\textsubscript{15:1}, and C\textsubscript{17:0} acids along with even chain acids. About one-half of BL at 346 and 750 m were comprised of odd-carbon numbered diacyl chains.

256 m, and < 7% for depths 270 m and deeper. Relative abundances of the major IPL classes are plotted in Fig. 7.

![Fig. 6. Depth profiles of: (a) total IPL; (b) IP-GDGT; (c) total BHP; and (d) total ladderane lipids. Note that IPL are µg L\(^{-1}\) but IP-GDGT, BHP and ladderane lipids are ng L\(^{-1}\).](Image 44x401 to 292x542)

![Fig. 7. Distributions of major IPL classes (relative percentage to total IPL) (see text for abbreviations).](Image 44x588 to 292x735)

![Abundances of major IPL classes (relative percentage to total IPL) (see text for abbreviations).](Image 44x598 to 292x735)
3.2.4. Ornithine lipids (OL) and glyceroceramides (Gly-Cer)

Ornithine lipids (XVI) were detected only in SPM within the deep chemocline and anoxic zones, constituting <0.5% of IPL except at 750 m where they were 3% of IPL. In OL, the α-amino group of ornithine is amide-linked to a 3-hydroxy acid and a second fatty acid is esterified to the 3-hydroxy group (3-OH used below). The core acyl pairs in the OL we found were identified as C14:0, C15:0 and C16:0.

Glycosylceramides (Gly-CER, IV), also called cerebrosides, belong to the class of sphingolipids that are characterized by a long-chain monounsaturated di-hydroxy amine structure (sphingosine). Gly-Cer, constituting ~3% of IPL, were observed only in the deep chemocline and in the anoxic sample. Core lipids of Gly-CER were predominantly composed of a 21–23 carbon sphinganine backbone with combinations of C14:0, C15:0 and C16:0 fatty acids.

3.2.5. Intact-glyco-glycerol dialkyl glycerol tetraethers (IP-GDGT)

IP-GDGT (containing intact polar glycol head groups XVI–XIX) were too low in abundance for reliable identification at 42 m in the Carico Basin, but a substantial concentration peak was observed between 245 and 256 m (Fig. 6b). We did not investigate intact phosphoglyco-GDGTs (e.g., Schouten et al., 2008) but they are potentially important archaeal IPL. Measured IP-GDGT concentrations were, for example, 2 ng L$^{-1}$ at 220 m (3% of IPL), 40 ng L$^{-1}$ at 256 m (3% of IPL) and 1 ng L$^{-1}$ at 750 m (0.6% of IPL). IP-GDGTs consisted of mono- and diglycosyl-GDGTs (1Gly-GDGT and 2Gly-GDGT, respectively; Fig. 9). 1Gly-GDGT comprised of crenarchaeol and lesser amounts of GDGT-0–GDGT-3 dominated the upper and deep chemocline zones (Fig. 9). 2Gly-GDGT were restricted to the upper and core chemoclines and were comprised of GDGT-0–GDGT-3 with lesser amounts of crenarchaeol. IP-GDGT also contained a compound previously termed H341-GDGT (for its 341 dalton loss during MS 2, Lipp and Hinrichs, 2009) that is now is thought to be a hydroxylated GDGT, 2Gly-OH-GDGT, restricted to the upper and core chemoclines and was comprised of crenarchaeol and lesser amounts of GDGT-0–GDGT-3. Overall, 2Gly-OH-GDGT appears to constitute up to 35% of IP-GDGT in the deep chemocline and in the anoxic sample. Mass spectra for 2Gly-OH-GDGT restricted to the upper and core chemoclines and were comprised of GDGT-0–GDGT-3 with lesser amounts of crenarchaeol. IP-GDGT also contained a compound previously termed H341-GDGT (for its 341 dalton loss during MS 2, Lipp and Hinrichs, 2009) that is now is thought to be a hydroxylated GDGT, 2Gly-OH-GDGT, restricted to the upper and core chemoclines and was comprised of crenarchaeol and lesser amounts of GDGT-0–GDGT-3. Overall, 2Gly-OH-GDGT appears to constitute up to 35% of IP-GDGT in the deep chemocline, and remarkably may represent 80% of IP-GDGT at 750 m.

LC-ESI-MS analyses of IP-GDGT do not provide full identification of the distributions of GDGT core lipids in the polar...
molecules. Thus, qualitative relative ring distributions of GDGT core lipids were determined by LC-APCI-MS. Core GDGT ranged from acyclic GDGT-0 to tricyclic GDGT-3 and pentacyclic GDGT, assumed to be crenarchaeol (Fig. 10). Crenarchaeol was consistently most abundant, ranging from 40% to 78% of core GDGT, with the lowest abundances in the core chemocline region. GDGT-0 increased in abundance with depth up to 30% of core GDGT. Abundances of GDGT-1 and GDGT-2 remained relatively constant over the depth profile. Interestingly, ring distributions obtained by LC-APCI-MS are not always the same as those inferred from LC-ESI-MS spectra; for example the dominance of crenarchaeol among core GDGT at all depths contrasts with the highly variable abundances of crenarchaeol in IP-GDGT. Whether this is a real pattern or simply an analytical artifact remains to be demonstrated.

3.3. Bacteriohopanepolyols (BHP)

Total BHP concentrations ranged between ~1 and 20 ng L$^{-1}$ (Fig. 6c), also being highest in the core chemocline. Of the four BHP we measured, bacteriohopanetetrol (17β,21β(H)-bacteriohopane-32,33,34,35-tetrol BHT XV) was the most abundant in the euphotic zone and the oxycline (65% of BHP; Fig. 11), its abundance decreased in the core and deep chemoclines (30% of BHP), but increased again in the deep anoxic zone (60% of BHP). Decreasing abundances of BHT were accompanied by increasing
abundances of what we term BHT I, proposed based on its chromatographic behavior (LC–MS and high temperature GC–MS) and mass spectrum to be a stereoisomer of BHT. Whereas together, [BHT + BHT – I] were relatively constant (~70% of BHP) at all depths investigated, BHT:BHT I ratios varied with depth, being, for example, 36 at 42 m, 5.7 at 220 m, 0.9 at 245 m and 256 m and 3.6 at 346 m. BHpentol (bacteriohopane-31,32,33,34,35-pentol XVI) was most concentrated in the euphotic zone (but only 0.3% of BHP) and quickly decreased in concentration and relative abundance with increasing depth. Aminotriol (17β,21β[1H]-35-aminobacteriohopane-32,33,34-triol XVII) had its highest relative concentration at 270 m and comprised 40–50% of BHP throughout the core and deep chemoclines.

3.4. Ladderane fatty acids

Ladderane fatty acids were abundant in the core chemocline (Fig. 6d), ranging from barely detectable down to 236 m and below 346 m to a maximum of ~7 ng/L at 245 m depth (Fig. 4c). They consisted of C18 and C20 fatty acids containing either 3- or 5-linearly concatenated cyclobutane rings (Sinninghe Damsté et al., 2002a), i.e., C18-[5]-, C20-[5]-, C18-[3]-, and C20-[3]- fatty acids (XVIII–XXI). Despite significant concentration changes down the water column for total ladderanes, there was little variation in the relative distributions of the four core ladderanes (data not shown). The C18-compounds together constituted up to ~85% of total ladderanes (of which the C18-[5]-ladderane fatty acid and C18-[3]-ladderane fatty acid were ~60% and ~20% of total ladderanes, respectively) whereas the remaining ~20% were the C20-compounds (with C20-[3]-ladderane fatty acid the more abundant).

3.5. Fatty acids (FA)

Concentrations of total FA (Fig. 12), calculated by summing the major components (Fig. 13), paralleled the depth profile of IPL (since GL and PL are the major sources of FA), being highest at 42 m (1300 ng FA/L−1) and with a secondary maximum at 245 m (590 ng L−1), and low in the upper chemocline (100 ng L−1) and deep anoxic zone (45 ng L−1). FA at 42 m (the euphotic zone) were typical of eukaryotes: C14:0, C16:1o5, C16:0, C18:1o9c, C18:4o3t, C18:3o3t, C20:5o3t, and C22:6o3t (Fig. 13). High abundances of C18:4o3t, C18:3o3t, C20:5o3t, and C22:6o3t PUFA probably derive from Gly-DAG and PC-DAG, as inferred from the LC–MS analysis. In contrast, FA within the core chemocline were highly enriched in C16:1o7t (XXII) and the unusual C16:1o7c (XXIII), together comprising ~50% of FA. In addition, a series of C15 to C20 mid-chain methoxylated fatty acids with methoxy groups variously located on carbons 8, 9, 10, 11, and 12 accounted for ~25% of FA; 9-methoxy-C16:0 and 10-methoxy-C16:0 (Fig. 12 XXIV and XXV) were the major FA methoxy FA (see Wakeham et al. (2010) for a detailed description of their identification and distributions). We speculate that the FA distributions in the core chemocline derive primarily from PE/PME/PDME-DAG that dominate the IPL in this depth interval. Odd-carbon numbered methyl-branched (iso- and anteiso-C15, and C17) fatty acids increased progressively in abundance beginning in the core chemocline through the 750-m anoxic sample, where anteiso-C15 was the second-most abundant FA. LC–MS interpretations indicated that odd-chain FA within the chemocline were present in both BL and PL fractions.

Several FA in the core and deep chemoclines, especially C16:1o7t and C16:1o7c, and to a lesser extent, methoxy FA, CH3O-C16:0 and CH3O-C18:0, were depleted in 13C relative to their δ13C values in both shallower and deeper depths (Fig. 13). δ13C values for C16:1o7c and C16:1o7t were particularly low in the core and deep chemoclines, −51.5‰, and −37.7‰, respectively. δ13C values for CH3O-C16:0 were as low as −36.0‰.

3.6. Apolar lipids

Depth distributions of the hopanoid hydrocarbon, diploptene (XXVI), and the isoprenoid hydrocarbons, squalene (XXIX), PMI (XXVIII), and lycopane (XXX), were similar: low concentrations in the euphotic zone, upper and core chemoclines but increasing concentrations in the deep chemocline (Fig. 12). The non-hopano- nid pentacyclic triterpenoid, tetrahymanol (gammaracar-3β-ol XXVII) was most concentrated in the core chemocline zone (Fig. 12).
4. Discussion

4.1. Biogeochemical zonation within the chemocline

The 6–15 m sampling intervals we targeted during CAR-139 should allow for observation of fine-resolution patterns across the chemocline. The unique feature of the resulting synoptic data set is that it encompasses water column chemistry, microbial compositions and multiple lipid biomarker classes, including POC, TN, C/N(a), δ13CPOC, nutrients, sulfur species, microbial distributions, IPL, BHP, ladderanes, FA and free apolar compounds. Hierarchical cluster analysis indicates that the water column we sampled partitions into five biogeochemical zones (Fig. 14): (i) the euphotic zone (42 m sample), (ii) an “upper chemocline” (also referred to as the oxycline; 205, 220, 236 m), (iii) a ~25 m thick “core chemocline” (the suboxic zone: 245, 256, 270 m), (iv) a “deep chemocline” (upper anoxic zone; 276, 296, 324, 346 m) and (v) the “deep anoxic zone” (750 m). Non-metric multidimensional scaling (NMDS) ordination analysis shows the relationships between geochemical species, microbial groups and lipid biomarkers in these 5 zones (Fig. 15).

(i) The euphotic zone sample (42 m) was collected at the fluorescence maximum where dissolved oxygen was still near its sea surface level and algal biomass was highest. Nitrate concentration (and phosphate, not shown) was low due to utilization by phytoplankton. Bacteria were 25-fold more abundant than archaea; aerobic heterotrophy was the dominant prokaryotic metabolism. IPL concentrations were high due to abundant glycolipids. IP-GDGT, BHP, and ladderane concentrations were low or below detection limit. Gly-DAG, 2Gly-DAG, SQ-DAG and polyunsaturated DAG project together in NMDS space with the 42 m depth (Fig. 15).

(ii) The upper chemocline (205, 220, and 236 m samples) is the transition between the oxygenated aphotic zone and the underlying suboxic zone. OM decomposition released NH4+ and reduced dissolved oxygen concentrations to below detection limits by ~240 m, fueling nitrate and nitrite production by nitrifiers which are consumed at the base of this layer by denitrifiers and anammox. Prokaryote numbers and rates of both heterotrophy and chemosynthesis decreased relative to the euphotic zone. POC and TN concentrations were low and NO2+ showed a weak secondary concentration maximum. NO3− had its highest concentration and ammonium was first detected. S0/C0 showed a sharp concentration peak, S2O3−/C0 and SO4−/C0 concentrations increased slightly, and sulfide (and CH4) was first detected. Cell numbers for bacteria (e.g., β- and ε-proteobacteria and anammox bacteria) and archaea were enhanced; rates of chemosynthesis and heterotrophy were elevated, with chemosynthesis outpacing heterotrophy. POC concentrations and beam attenuation increased, C/N(a) decreased, and POC became more 13C-depleted. Concentrations of IPL, IP-GDGT, BHP, ladderanes and fatty acids (especially the methoxy-acids) all peaked sharply. Phospholipids, predominately PE/PME/PDME-DAG, replaced glycolipids and betaine lipids as dominant IPL. The sharp peak for tetrahymanol probably arose from bacteriovorous ciliates grazing on chemosynthetic biomass which might be low in sterols (Conner et al., 1968; Harvey and McManus, 1991). Nitrite, elemental S and thiosulfate, PE/PME/PDME-DAG, 16:1ω7t, CH3O-16 and CH3O-18, C18 [5]-, and C18 [3]-ladderane FA, BHT 1, 2Gly-OH-GDGT, 1Gly-GDGT(cren) and 2Gly-GDGT (cren) and 2Gly-GDGT (0–3 rings) all are associated with the core chemocline depths on the NMDS plot. The core chemocline (245, 256, and 270 m samples) was suboxic with O2 and H2S near detection limits. NO3− showed a weak secondary concentration maximum, NO2+ had its highest concentration and ammonium was first detected. S0 showed a sharp concentration peak, S2O3− and SO4− concentrations increased slightly, and sulfide (and CH4) was first detected. Cell numbers for bacteria (e.g., β- and ε-proteobacteria and anammox bacteria) and archaea were enhanced; rates of chemosynthesis and heterotrophy were elevated, with chemosynthesis outpacing heterotrophy. POC concentration and beam attenuation increased, C/N(a) decreased, and POC became more 13C-depleted. Concentrations of IPL, IP-GDGT, BHP, ladderanes and fatty acids (especially the methoxy-acids) all peaked sharply. Phospholipids, predominately PE/PME/PDME-DAG, replaced glycolipids and betaine lipids as dominant IPL. The sharp peak for tetrahymanol probably arose from bacteriovorous ciliates grazing on chemosynthetic biomass which might be low in sterols (Conner et al., 1968; Harvey and McManus, 1991). Nitrite, elemental S and thiosulfate, PE/PME/PDME-DAG, 16:1ω7t, CH3O-16 and CH3O-18, C18 [5]-, and C18 [3]-ladderane FA, BHT 1, 2Gly-OH-GDGT, 1Gly-GDGT(cren) and 2Gly-GDGT (cren) and 2Gly-GDGT (0–3 rings) all are associated with the core chemocline depths on the NMDS plot.

(iv) The deep chemocline (276, 296, 324, and 346 m samples) is the transition between the suboxic core chemocline and the strongly euxinic deep basin and was characterized by increasing concentrations of H2S, S2O3−, SO4−, NH4+, and CH4. Prokaryote numbers and chemosynthetic production remained high. IPL concentrations, dominated by PE/PME/PDME-DAG, were lower than in the core chemocline; OL and Gly-CER first appeared. Abundances of methoxy-FA decreased but odd-carbon, methyl-branched FA increased. Diploptene,
PMI, squalene and lycopane concentrations were highest in the deep chemocline. Thus sulfite and phosphate, DPG, lyso-DPG, DEG-PE/PME, OL, and odd-BL, aminotriol, PMI, anteiso-C<sub>15</sub> FA, 1Gly-GDGT (0–3 rings) plot together in NMDS space. Lycopane, squalene and diploptene plotted between the shallow anoxic depths and the deep anoxic sample. 

(v) The anoxic zone (750 m sample) was strongly euxinic (~40 µM H<sub>2</sub>S, not plotted below 450 m in Fig. 3). POC
Glycolipids constitute up to 80% of polar lipid in thylakoid membranes of chloroplasts of photoautotrophs (Nishihara et al., 1980; Kates, 1990; Wada and Murata, 1998; Van Mooy and Fredricks, 2010). They are less common in bacteria (Siegenthaler, 1998; Benning, 1998; López-Lara et al., 2003; Hötzl and Dörmann, 2007). Acyl chains of GL are rich in C14:0, C16:0, C18:1, C18:2, and C20:5 PUFA. Gly-DAG disappeared quickly with depth in the Cariaco Basin, probably because algal detritus is not an important component of slowly sinking SPM below the euphotic zone. Zooplankton grazing may package GL into rapidly sinking fecal pellets which we did not analyze. Glycosidic and fatty acyl bonds are also rapidly hydrolyzed after cell death (Harvey et al., 1986; Matos and Pham-Thi, 2009).

Ratios of Gly-DAG to 2Gly-DAG and SQ-DAG vary among different algal species (Kates, 1990; Benning, 1998; Van Mooy et al., 2006, 2009). Gly-DAG predominate in eukaryotes but SQ-DAG can be abundant in cyanobacteria. There are few reports on molecular compositions of intact glycolipids in oceanic surface water SPM. Van Mooy and Fredricks (2010) found concentrations of Gly-DAG~SQ-DAG~2Gly-DAG in the oligotrophic eastern subtropical South Pacific Ocean and suggested a predominately Prochlorococcus source for Gly-DAG and SQ-DAG. A subsequent study by Popendorf et al. (2011) used 13C-isotope probing and cell sorting flow cytometry to indicate cyanobacteria as the major source of Gly-DAG and SQ-DAG in the western North Atlantic Ocean. In the Cariaco Basin, however, diatoms rather than cyanobacteria are the major phytoplankton throughout the year (Lorenzoni et al., 2011). The dominance of Gly-DAG over SQ-DAG probably reflects a greater input to Cariaco Basin SPM from eukaryotic than prokaryotic plankton.

Phospholipids (PL) are often less abundant within algal polar lipids than GL. (Ratledge and Wilkinson, 1988). PC-DAG are important in the lipid bilayer of the chloroplast membrane of eukaryotic and prokaryotic photosynthetic bacteria (Harwood, 1998; Wada and Murata, 1998; Van Mooy et al., 2009; Popendorf et al., 2011). But in the Cariaco Basin euphotic zone they are of low abundance.

BL play a metabolic role in the desaturation and redistribution of fatty acids among cellular lipids (Eichenberger et al., 1996). A wide variety of phytoplankton and bacteria are potential BL sources (e.g., Synechococcus WH8102, Synechococcus WH7803, Synechococcus WH5701, Prochlorococcus MED4, Crocosphaera watsonii, Trichodesmium erythraeum, Thalassiosira pseudonana, Chaetoceros affinis, Emiliania huxleyi, Rhodobacter sphaeroides, Bacillus subtilis; Benning et al., 1995; Dembitsky, 1996; Kato et al., 1996; Künzler and Eichenberger, 1997; Riekhof et al., 2005; Khozin-Goldberg and Cohen, 2006; Van Mooy et al., 2006, 2009). That BL in the euphotic zone of the Cariaco Basin are likely derived from eukaryotic algae is reinforced by the dominance of BL in even numbered acyl carbon chains comprised of predominately C14:0, C16:0, C16:1, C18:1, C18:2, and C22:0 moieties. In other regions where cyanobacteria dominate over eukaryotic algae, the major source of BL may be heterotrophic bacteria (Van Mooy and Fredricks, 2010; Popendorf et al., 2011; see Section 4.2.2).

The structures and charge distributions of SQ-DAG vs. PG-DAG and of BL vs. PC-DAG, respectively, are similar, suggesting interchangeability. SQ-DAG and BL can be non-phosphorus substitutes for phospholipids in P-deficient environments (Benning et al., 1995; Van Mooy et al., 2009; Popendorf et al., 2011) where they are preferentially biosynthesized over PL as membrane constituents and/or intermediates in cellular metabolism. In the eastern subtropical South Pacific where phosphate concentrations were low, PG-DAG and PC-DAG concentrations were low and SQ-DAG and BL concentrations were high (Van Mooy and Fredricks, 2010). However, neither SQ-DAG nor BL were strongly correlated with phosphate, leading to the suggestion that in addition to phosphate limitation, variations in community structure might be equally important in determining relative proportions of SQ-DAG vs. PG-DAG and BL vs. PC-DAG. In the western North Atlantic there were considerable variations between IPL compositions of high vs. low phosphate samples and between Synechococcus and Prochlorococcus (Popendorf et al., 2011). In surface waters of the Cariaco Basin, phosphate was low (<0.1 μM at the fluorescence maximum; see also CARIACO data http://ocb.whoi.edu/jg/dir/OCB/CARIACO/). But BL, even at low absolute concentrations, constituted a significant proportion of IPL whereas PL and SQ-DAG did not, consistent with a predominately eukaryotic photo-plankton community in the analyzed SPM size fraction.

4.2.2. Bacteria

Bacterial membrane lipids are dominated by BL and PL, making these lipids the most abundant bacterial lipids in the Cariaco Basin SPM, especially in the chemocline where bacterial numbers are high. Recent studies have also shown that heterotrophic bacteria are capable of biosynthesizing some Gly-DAG (Popendorf et al., 2011; see
also Benning et al., 1995). Unfortunately the ubiquity and relatively limited structural diversity of BL and PL across a wide range of metabolic functions restrict their diagnostic utility. The structurally more unique bacteriohopanepolyols and ladders are that also produced by bacteria represented only a small fraction of bacterial biomarkers in the Cariaco Basin but offer a considerably improved diagnostic potential in the suboxic zone (discussed below). Ornithine lipids (OL) and glycosylceramides (Gly-CER) were minor IPL and might be derived from Gram-negative bacteria (Olsen and Jantzen, 2001; Geiger et al., 2010). OL may be specifically derived from sulfate-reducing bacteria (Makula and Finnerty, 1975; Schubotz et al., 2009).

BL are synthesized by α-proteobacteria (R. sphaeroides and Sinorhizobium meliloti) during phosphate limiting conditions (Benning et al., 1995; Geiger et al., 1999), and the genes responsible for BL synthesis are restricted to some orders of α-proteobacteria (Rhodobacterales, Sphingomonadales, Rhizobiales and members of the Planctomycetes) (López-Lara et al., 2003; Geiger et al., 2010). In Cariaco Basin SPM, BL and PL containing odd-carbon acyl moieties (Table 1) are consistent with bacterial sources (Olsen and Jantzen, 2001; Geiger et al., 2010). BL have not been well characterized in deep-sea bacteria, but they are important IPL in the suboxic and anoxic Black Sea (Schubotz et al., 2009).

PL are key membrane lipids in bacteria (cf. Dowhan, 1997; Zhang and Rock, 2008; Popendorf et al., 2011). PE/PME/PDME-DAG are the major PL in the Cariaco Basin. They are biosynthesized by nitrogen-fixing bacteria (Azobacter agilis), nitrifying bacteria (Hyphomicrobium vulgare), and nitrate-reducing bacteria (Paracoccus denitrificans), sulfate-oxidizing bacteria (Thiobacillus thiooxidans, T. novellus, T. intermedius, T. neapolitanus, and T. thioides), sulfate-reducing bacteria (Desulfococcus multivorans, Desulfomicrobium sp., Desulfobacter postgutai, Thermodesulfobacterium commune, Desulfosarcina variabilis), and methanotrophic bacteria (Methyllococcus capsulatus, Methanosinus trichosporium, Methylophilus methanica, M. rubra, Methanocella paludata) (Barridge and Shively, 1968; Goldfine and Hagen, 1968; Makula, 1978; Goldfine, 1984; Ratledge and Wilkinson, 1988; Fang and Barcelona, 1998; Fang et al., 2000; López-Lara et al., 2003; Rütters et al., 2002; Sturt et al., 2004). PE-DEG and PE-AEG phospholipids may also be derived from sulfate-reducing bacteria since they are present in cultured SRB (D. multivorans, Desulfomicrobium sp., D. postgutai, T. commune, D. variabilis; Zhang et al., 2003) and sulfate-reducing bacteria (Methanocella palustre, Methanocella palustre, M. rubra, M. capsulatus, M. capsulatus, M. capsulatus) and sulfur-oxidizing bacteria (Thiobacillus, Beggiatoa and Thioploca; Kegler et al., 1986; McCaffrey et al., 1989; Zhang et al., 2005) or in environmental samples where SRB are important (Hiirichs et al., 2000; Pancost et al., 2001, Rossel et al., 2008; Schubotz et al., 2011). Analysis during this investigation of isolates of sulfur-oxidizing bacteria from the Cariaco Basin revealed that PE-DAG were slightly more abundant than PG- or DPG-DAG. All three PL classes in the isolates contained high proportions of C16:1ω7c, C16:1ω7t, and mid-chain-methoxylated FA. Branched odd-chain FA are not very abundant in cultured sulfur-oxidizers (Thiobacillus, Beggiatoa and Thioploca; Kegler et al., 1986; McCaffrey et al., 1989; Zhang et al., 2005) or in the sulfur-oxidizer isolates. Rather, their sources in the core chemo-Selective oxidase (Geoarchaeum metallireducens and Shewanella algae; Zhang et al., 2003) and sulfate-reducing bacteria (Desulfosarcina/Desulfococcus group, Desulfobacter sp., Desulfobulbus sp., D. multivorans, Desulfosarcina variabilis; Taylor and Parkes, 1983; Parkes and Taylor, 1983; Dowling et al., 1986; Makuda, 1991; Kohring et al., 1994; Elvert et al., 2003).

Ornithine lipids (OL) are widespread as membrane lipids among Gram-negative bacteria but are absent from eukaryotes and archaea (Ratledge and Wilkinson, 1988; Asselineau, 1991; López-Lara et al., 2003; Geiger et al., 2010). Potential OL sources within the chemo-Selective oxidase of the Cariaco Basin include nitrate-reducers (P. denitrificans; Thie et al., 1980), sulfur-oxidizers...
Hopanoids have traditionally been thought to serve a rigidifying function in bacterial membranes (Rohmer et al., 1984; Ourisson et al., 1987), similar to steroids in eukaryotes. Recent work, however, shows much more diverse physiological functions, including alterations of membrane structure in response to changes in temperature, dessication, pH, and in cellular differentiation (Kannenberg and Poralá, 1999; Welander et al., 2009; Doughty et al., 2009, 2011). BHP biosynthesis is not universal among prokaryotes (Rohmer et al., 1984; Pearson and Rusch, 2009), however BHP have been identified in cyanobacteria, purple non-sulfur bacteria, acetoc acid bacteria, methano- and methylotrophs, and nitrifying, anammox and sulfate- and metal-reducing bacteria (reviewed by Talbot and Farrimond, 2007). They do not appear to occur in Bacillus and are absent from all Archaea. The depth profile of BHP in the Cariaco Basin corresponded with the profile of bacterial numbers, but concentrations were low compared to other lipids.

BHT is the most common BHP in cultured bacteria and in environmental samples (Talbot et al., 2003c, 2007a, b; Talbot and Farrimond, 2007), and BHT in the euphotic zone of the Cariaco Basin may derive from cyanobacteria (Talbot and Farrimond, 2007). Methylation at C-2 is widely considered specific for cyanobacteria. Yet 2-methyl BHP are generally not known in marine species (Summons et al., 1999; Talbot et al., 2008) and we did not observe them here. BHpentol, with its highest concentration in the photic zone, has also been identified in some cyanobacteria (e.g. Neunlist et al., 1985, 1988; Talbot et al., 2007a, b). BHT I and aminotriol increased proportionally with depth in the chemocline; both might be biosynthesized by sulfate-reducing bacteria (Desulfovibrio sp.; Blumenberg et al., 2006). BHT I, or a compound with similar chromatographic properties, has also been reported in other low oxygen/anoxic water columns and sediments from the Peru, Arabian Sea and Benguela upwelling systems (Watson, 2002; Blumenberg et al., 2010; Sáenz et al., 2011). Sáenz et al. (2011) suggest that BHT I is selectively produced in oxygen-deficient environments. Incorporation of a side chain biosynthetic precursor such as S-adenosyl methionine (Bradley et al., 2010; Rohmer et al., 1989) could isomerize BHT to BHT I, or a post-biosynthetic microbially mediated isomerization of BHT could yield BHT I. In strongly suboxic environments such as the Peru upwelling and Cariaco Basin, BHT I is as abundant as BHT. Some BHT in the core chemocline of the Cariaco Basin might also derive from anammox bacteria (Sinninghe Damsté et al., 2004, 2005; Rattray et al., 2008).

BHP with NH₂ functionality at C-35 are considered biomarkers for aerobic methane oxidizing bacteria (Neunlist and Rohmer, 1985a, b; Talbot et al., 2001; Blumenberg et al., 2004, 2007). Among amino-BHP, only aminotriol was detected in the Cariaco Basin (perhaps surprising since aminotriol and aminopentol are considered better indicators of aerobic methanotrophy), but it accounted for up to 50% of BHP within the chemocline. Only a thin layer of aerobic oxidation of methane occurs in the suboxic zone of the Cariaco Basin (Ward et al., 1987), and our sampling profile could have missed it; most oxidation of methane occurs anaerobically in the deep anoxic zone.

Diploptene is found at low levels in many hopanoid-producing bacteria (e.g. Rohmer et al., 1984; Bradley et al., 2010) as it is a biosynthetic intermediate in the pathway to BHP, and is present in oxygen-depleted environments (Wakeham, 1990; Elvert et al., 2001; Wakeham et al., 2007). But in the Cariaco Basin, diploptene’s concentrations were very low and, notably, its concentration depth profile was quite different from that of intact BHP. Its peak localization at depths different from the peak of BHP suggests that there are multiple sources and sinks for the C₃₀ hopanoids and their extended counterparts in the Cariaco Basin chemocline.

Multiple lines of evidence – co-existing nitrite and ammonium required for anammox, the sharp peak in anammox bacteria (AMX-positive cells), and the sharp peak of ladderane lipids – indicate that anammox occurs within the core chemocline. Anammox is restricted to a phylogenetic group of Brocadiales related to the order Planctomycetales, a distinct division of Bacteria which is phylogenetically as distant from Proteobacteria as from Archaea (Strous et al., 1999; Chistoserdova et al., 2004). Ladderane lipids containing hydrocarbon chains of linearly condensed cyclobutane rings are components of the membrane of the specialized organelle, the anammoxosome, in which the anammox process takes place (e.g., “Candidatus Anammoxoglobus propionicus”, “Ca. Brocadia fulgida” and “Ca. Kuenenia stuttgartiensis”, and “Ca. Scalindua spp.”). The core ladderane hydrocarbon chains are usually linked to a glycerol backbone with PC, PE of PG head groups (e.g., Boumann et al., 2006; Jaeschke et al., 2009), but here we only determined the core ladderane fatty acids after hydrolysis of the extract. In view of the low concentrations of AMX-positive cells and ladderane fatty acids it is unlikely that IP-ladderane lipids would be identified using our general IP-identification method. Rattray et al. (2010) observed a temperature effect on the relative abundances of core C₂₀ [S]-ladderane fatty acids vs. C₁₈ [S]-ladderane fatty acids in enrichments of “Ca. A. propionicus”, “Ca. B. fulgida” and “Ca. K. stuttgartiensis”, “Ca. Scalindua spp.” and field samples, and Boumann et al. (2009) recently demonstrated that chain-length adaptation (C₂₀ vs. C₁₈) in core ladderanes affects lipid packing density as a control on membrane function at different growth temperatures in “Ca. K. stuttgartiensis”, “Ca. B. fulgida” and “Ca. Scalindua spp.”. We did not observe a significant variation in distributions of ladderane lipids as a function of depth in the Cariaco Basin, but this may be due to the fact that anammox is restricted to the core chemocline in which there was little temperature variation, and ladderane lipids detected at other depths may in fact have originated within the core chemocline.

4.2.3. Archaea

Concentrations of IP-GDGT showed a strong concentration peak in the core chemocline, following elevated archaeal cell numbers. IP-GDGT in the upper and deep chemocline zones were comprised exclusively of monoglycosidic polar head groups but IP-GDGT in the core chemocline were both monoglycosyl- and diglycosyl-GDGT, suggesting multiple archaeal sources that vary with depth and redox. We do not know the structures of the glycosidic head groups in our SPN samples, but a compilation of IP-GDGT shows glucose to be most common, in addition to galactose, mannose, galactose and modified sugars such as (N)-acetylglucosamine (Koga and Morii, 2005; Koga and Nakano, 2008; Lin et al., 2010). The tentatively identified 2Gly-OH-GDGT (X.-L. Liu, personal communication) was abundant in the core chemocline and was the major IP-GDGT in the anoxic SPN sample. Several studies had previously reported novel but not fully identified GDGT that are now believed to be hydroxylated in 

(Thiobacillus thiooxidans; Shively and Knoche, 1969; Knoche and Shively, 1972; Dees and Shiveley, 1982; Thiele et al., 1984), and some sulfate-reducers (Desulfovibrio gigas but not Desulfuricans or Des. vulgaris; Makula and Finnerty, 1975). OL consist of both amide- and ester-bound FA; only ester-linked FA were determined in the present study. In D. gigas, the dominant ester-linked FA in OL (and PG-DAG and PE-DAG) was anteiso-C₁₅, and anteiso-C₁₅ was the second most abundant FA in the anoxic SPM. Among glycosylceramides (Gly-CER), phosphosphingolipids are relatively common in Gram-negative anaerobic bacteria, e.g. in the Bacteroides group (Kato et al., 1995; Olsen and Jantzen, 2001). Glycosphingolipids are only ubiquitous in the
particulate matter in the suboxic Black Sea (Schubotz et al., 2009) and sediments (Lipp and Hinrichs, 2009; Liu et al., 2011). Schouten et al. (2008) described a GDGT the mass spectrum of which is consistent with a hydroxylated GDGT in the thaumarchaeon “Candidatus Nitro-sopumilus maritimus” [the term “Thaumarchaeota” is now used to denote mesophiliic archaea formerly known as Marine Group I Crenarchaeota (Brochier-Armanet et al., 2008; Spang et al., 2010)], and (X.-L. Liu, personal communication) has proposed several hydroxylated GDGT to be in the methanogenic euryarchaeon Metha-nococcus thermolithotrophicus.

In addition to depth-related variations in proportions of Gly-GDGT and 2-Gly-GDGT among IP-GDGT, there were variations in the ring distributions of core lipids. GDGT-0 and crenarchaeol were the dominant core GDGT throughout the water column. GDGT-0, comprised only of acyclic bisphenyls, is often the dominant GDGT in euryarchaeota (see compilation in Schouten et al., 2007) and cultured thaumarchaeota (Cenarchaeum symbiosum, “Ca. Nitrospumilus maritimus; Delong et al., 1998; Sinninghe Damsté et al., 2002c; Schouten et al., 2008). Crenarchaeol is unique among GDGT by having a cyclohexyl moiety in addition to four cyclopetane rings (Sinninghe Damsté et al., 2002b) and is often the most abundant GDGT in marine water column particles and sediments (e.g., Wuchter et al., 2005; Wakeham et al., 2007; Turich et al., 2007). It is has also been found in hot springs and in cultured thermophilic ammonium oxidizing crenarchaeota (e.g., “Ca. Nitroscoccus yellow-storii,” “Ca. Nitrososphaera gargensis”; Pearson et al., 2004; Schouten et al., 2007; de la Torre et al., 2008; Pitcher et al., 2009, 2010) and soils (Leininger et al., 2006; Weijers et al., 2006). A common feature of recent reports of crenarchaeol in cultured or enriched archaea and in environmental samples is its association with ammonium oxidizing archaea (AOA), leading to its being proposed as specific to AOA (de la Torre et al., 2008; Pitcher et al., 2010).

Interestingly, concentration profiles of squalene and PMI, presumably of archaeal origin, were offset from the concentration peak of their archaeal IP-GDGT analogs. Squalene and PMI have been identified in some methanogens (several species of Methanobacterium, Methanococcus, Methanospirillum and Methanosarcina; Tornabene and Langworthy, 1979; Holzer et al., 1979; Risatti et al., 1984; Schouten et al., 1997). Methanogenesis in the Cariaco Basin water column may be limited by the presence of sulfate (like the Black Sea, most methane is produced in the sediments; Reeburgh, 1996). Anaerobic methane oxidizing archaea also biosynthesize PMI (Elvert et al., 1999; Thiel et al., 1999; Hinrichs et al., 2000; Bian et al., 2001) but the bulk of AOM in the Cariaco Basin occurs deep in the anoxic zone below our 750 m sample (Ward et al., 1987). Lycopane, the provenance of which remains unresolved, had a depth profile similar to squalene and PMI. Suggested sources for lycopane have ranged from lycopene or carotenoids of phototrophic algae to methanogenic archaea (Wakeham et al., 1993, 2007), but head-to-head linkages of biphayte moieties in lycopane are distinct from tail-to-tail linkages of archaeal GDGT. Nonetheless, preservation of lycopane, whatever its origin, is considered an indicator of oxygen-depleted environments (Sinninghe Damsté et al., 2003), and redox boundaries such as the Cariaco Basin (and Black Sea; Wakeham et al., 2007) may be important in this respect.

5. Sulfur and nitrogen cycling, biomarkers and chemoaotrophy within the chemocline

Overlaps in oxidation states of N and S species in the Cariaco water column are well documented in the long-term records of CARIACO (Scranton et al., 2001, 2006; Ho et al., 2004) and represent competing oxidation and reduction processes along the redox gradient, possibly including effects of occasional lateral intrusions of oxygenated waters at chemocline depths (Astor et al., 2003). Microbiologically mediated redox reactions involving S are potentially significant. CARIACO investigations suggest that the major pathway of organic carbon oxidation in the redoxcline is not sulfate reduction but rather reduction of intermediate oxidation states of S or metal oxides (Ho et al., 2004). Intermediate oxidation states of sulfur – $S_2O_3^{2-}$, $SO_3^{2-}$ and particulate S$^0$ – have been implicated as key substrates supporting chemoauto trophic production (Taylor et al., 2001, 2006; Hayes et al., 2006; Percy et al., 2008; Li et al., 2008) at a level that often surpasses the flux of phototrophic primary production from surface waters to the chemocline (Thunell et al., 2000; Taylor et al., 2001; Li et al., 2012). The broad peaks of $\alpha$- and $\beta$-proteobacteria we observed are indicative of chemoautotrophic sulfur-oxidizing bacteria. Thiosulfate-oxidizing $\alpha$- and $\beta$-proteobacteria, such as Thiobacillus sp., Thiomicrospira denitrificans and Sulfurimonas spp. have been identified within the chemocline of the Cariaco Basin (Tuttle and Jannasch, 1973; Jannasch et al., 1991; Madrid et al., 2001; Lin et al., 2008). These organisms are typically aerobic and denitrifying sulfur oxidizers. In contrast, within the redoxcline of the Gotland Basin of the Baltic Sea in the absence of nitrate, the dominant chemoautotrophic $\alpha$-proteobacterium, Thiomicrospira denitrificans, uses anaerobic manganese-dependent sulfur oxidation (Jost et al., 2010). Stable isotope (13C) probing (SIP) experiments in the Cariaco Basin have shown that $S_2O_3^{2-}$ was the best of the tested substrates ($S_2O_3^{2-}$, $SO_3^{2-}$ and particulate $S^0$) for chemoautotrophic production and biosynthesis of lipids by sulfur oxidizers in water collected from the suboxic zone (Wakeham et al., 2010). The major PL produced in these SIP experiments was PE-DAG with lesser amounts of DPG-DAG and PG-DAG (Wakeham, unpublished), and 16:1o7c, 16:1o7t, and mid-chain methoxy acids in the SIP experiments were highly enriched in 13C. Sulfate reduction is quantitatively minor with respect to sulfur cycling within the chemocline of the Cariaco Basin and low numbers of SRB (1–4% of prokaryotes) are present, in line with previous CARIACO studies (Lin et al., 2006, 2008). SRB are traditionally considered to be strict anaerobes, yet some are present in mildly oxygenated environments, and low numbers of SRB were present in the upper chemocline of the Cariaco Basin. In the Black Sea significant numbers of SRB of the genera Desulfomaculum, Desulfobrio and Desulfobacter have been reported in the oxic zone (30 m) and Desulfomicrobium is prevalent in the suboxic zone (150 m) (Bryukhanova et al., 2011). As mentioned above, PE-DEG phospholipids in (and below) the suboxic zone of the Cariaco Basin could derive from SRB.

Diverse nitrogen cycling processes by microbes also occur in the core chemocline of the Cariaco Basin. Nitrate, nitrite and ammonium all approach zero at the bottom of the suboxic zone, indicating competing nitrification (oxidation of ammonium to $NO_3^-$ via $NO_2^-$), denitrification (reduction of $NO_3^-$ to $N_2$ via $NO_2^-$ at the expense of OM), and anammox (anaerobic oxidation of ammonium via $NO_2^-$ produced by denitrification). In suboxic environments, ammonium oxidizing archaea (AOA) and ammonium oxidizing bacteria (AOB) may coexist by competing for available ammonium diffusing into the suboxic zone from the underlying anoxic zone (Lam and Kuyper, 2011). AOA may out-compete AOB in environments where sulfur-oxidizing bacteria are present (Park et al., 2010).

Marine Thaumarchaeota may be either strict autotrophs or mixotrophs, using either a modified hydroxypropionate–hydroxybutyrate cycle for autotrophic carbon assimilation using CO$_2$ as a carbon source or the oxidative tricarboxylic acid cycle using organic carbon as carbon source (Hallam et al., 2006; Berg et al., 2010). They thus can function as light-independent chemoaotrophic nitrifiers, using reduced nitrogen such as NH$_4^+$ as an energy source and reducing nitrate to nitrite (Köneke et al., 2009).
2005; Wuchter et al., 2006; Martens-Habbena et al., 2009). Archaeal amoA genes encoding the ammonium monooxygenase α-subunit, the key enzyme also in aerobic AOB, are present in diverse ocean settings, including oxygen deficient regions (e.g., Francis et al., 2005; Wuchter et al., 2006; Coolen et al., 2007; Lam et al., 2007; Agogué et al., 2008; Beman et al., 2011; Pitcher et al., 2011a, b). Enrichment studies showing preferential growth of Thaumarchaeota over AOB at low oxygen tensions (Park et al., 2010) help explain the peak in IP-GDGT and the abundance of crenarchaeol and AOA in the OMZ of the Arabian Sea (Sinninghe Damsté et al., 2002c; Pitcher et al., 2011a, b) and in the suboxic Black Sea (Wakeham et al., 2007; Coolen et al., 2007).

Nitrifying Thaumarchaeota may a source of nitrite for the anammox reaction (Coolen et al., 2007; Lam et al., 2007). Cell numbers of anammox bacteria and ladderine fatty acids in the Cariaco Basin are limited to the suboxic zone, near the peak in numbers of anammox bacteria (230 m), ladderanes (245 m) and NO$_2^-$ maxima. Recent results from the Arabian Sea, on the other hand, show that anammox communities can also to function outside the NO$_2^-$ maximum (Pitcher et al., 2011a, b). In the Cariaco Basin, there is a slight offset in the depths of the anammox bacteria (230 m), ladderanes (245 m) and NO$_2^-$ (250 m), but it is uncertain whether this is a real spatial separation or an artifact of the week's time between respective sampling. The fact that anammox is most prevalent in the suboxic zone also fits with anammox bacteria also requiring oxygen, albeit at low levels. Anammox bacteria have been recovered from suboxic waters of the Benguela Current OMZ with 9 µM O$_2$ (Kuyper et al., 2005) but in culture anammox may be inhibited at concentrations as low as 1 µM O$_2$ (Strous et al., 1997). Anammox is also apparently inhibited by sulfide (Jensen et al., 2008), so that anammox in the Cariaco Basin is therefore restricted to the depth interval above which H$_2$S was first detected (260 m).

Anammox oxidation of ammonium and anammox in the Cariaco Basin thus appear to coexist in the narrow confines (~25 m) of the suboxic core of the chemocline. A similar coexistence apparently also occurs in the thin suboxic zone of the Black Sea (Lam et al., 2007; Wakeham et al., 2007). In contrast, however, the two metabolisms were segregated by ~400 m in the OMZ of the Arabian Sea (Pitcher et al., 2011a, b), most likely because the O$_2$ gradient is much less steep. Archaeal oxidation of ammonium in the Arabian Sea occurred in the oxycline at ~200 m depth where dissolved O$_2$ concentration was ~5 µM whereas anammox peaked in the core of the OMZ at ~400 m and ~1 µM O$_2$.

Depletions in stable carbon isotopes of POC at redox boundaries are frequently attributed to chemoaotrophic processes (e.g., Fry et al., 1991; Freeman et al., 1994; Hollander and Smith, 2001), but often without case-specific knowledge of the microbial community composition and function. In the Cariaco Basin, we now have comprehensive geochemical, biomarker, and microbial distributions that shed light on the major biogeochemical processes within the chemocline that may help explain the lower δ$^{13}$CPOC values within the chemocline. For example, sulfur-oxidizers such as Thiomicrospira sp., Thiothrix neapolitanus and Beggiatia sp. produce biomass that is ~25% depleted in $^{13}$C compared to its dissolved inorganic carbon (DIC) source (Nelson and Jannasch, 1983; Nelson et al., 1986; Ruby et al., 1987). Archaea use several autotrophic carbon fixation mechanisms. Thaumarchaeota using the modified 3-hydroxypropionate pathway may fractionate against HCO$_3^-$ by ~−12‰ (House et al., 2003; Berg et al., 2010). Anammox bacteria, e.g., “Ca. Brocadia anammoxidans” (Schouten et al., 2004) and the ammonium-oxidizing chemotrophic bacteria, Nitrosomonas europaee (Sakata et al., 2008) produced cells that were ~−20‰ depleted relative to CO$_2$. Freeman et al (1994) suggested that a δ$^{13}$CO$_2$ of ~−10‰ in the chemocline of the Cariaco Basin would yield chemotrophic biomass with δ$^{13}$C values between ~−39‰ and ~−22‰ depending on the specific carbon uptake mechanism and carbon flow pathways. These values bracket the δ$^{13}$CPOC we measured in the core chemocline of the Cariaco Basin (~−28‰), POC in the chemocline of the Cariaco Basin could also contain a component of Calvin-cycle photoautotrophic carbon that would be isotopically heavier, more like the ~−22‰ measured for surface plankton-derived SPM (F. Muller-Karger, personal communication). Del$^{13}$C values of specific lipids are typically even lower (Hayes, 2001), which may help explain the strong isotope depletions for 16:1o7c, 16:1o7t, and mid-chain methoxy acids relative to POC and other FA in the chemocline; isotopically-depleted FA have also been observed in Beggiatia-dominated sediments (Zhang et al., 2005). Previous measurements of δ$^{13}$C of ~−36‰ and ~−42‰ for diplopentane in the chemocline and sediment of the Cariaco Basin were also taken as indicative of chemotrophy (Freeman et al., 1994). Phytane and biphytanes derived from GDGT, however, had higher δ$^{13}$C values (~−32‰ for phytane and ~−22‰ for the biphytanes), and PMI and lycopane were both ~−25‰ (Wakeham et al., 2004). Odd carbon methyl-branched FA and ladderane FA of “Ca. Brocadia anammoxidans” were ~−20‰ and ~−47‰, respectively, more depleted in $^{13}$C than biomass and CO$_2$ (Schouten et al., 2004). Fatty acids and hopanoids of Nitrosomonas europaea were ~−10‰ and ~−20‰, respectively, depleted in $^{13}$C than biomass and CO$_2$, respectively (Sakata et al., 2008).

6. The fate of chemoaotrophic biomass in the Cariaco basin

Most chemotrophic OC appears to be recycled in the suboxic and upper anoxic zone rather than sinking (Li et al., 2012). Some is probably consumed by bacterivorous microzooplankton. “Mini-pellets” may be produced that contain intact bacteria (Gowing and Silver, 1985) but probably do not sink much (Turner, 2002). For the Cariaco Basin, Li et al. (2012) postulate the existence of a localized sulfur cycle in which sulfate reducers oxidizing chemotrophically fixed carbon produce sulfide that is subsequently utilized by chemotrophs for further carbon fixation. Evidence for this hypothesis is the mismatch between supply of oxidants and reductants and measured chemotrophic production rates. Neither diffusive flux from sulfidic waters below nor advective flux either vertically or laterally supplies sufficient sulfide for chemotrophy. Sulfate reduction in the suboxic zone may be an additional source of sulfide (Canfield et al., 2010), with both residual photoautotrophic OC from surface waters and new chemotrophic OC being utilized by sulfate reduction. Similar internal cycling may be common at other chemoclines and has been proposed for oceanic anoxic events (Meyer and Kump, 2008).

Suspended particulate biomarkers derived from water column chemotrophy may sink more efficiently if they are incorporated into larger particles, either by biological or physical aggregation. A microbe → protozoa → zooplankton microbial loop (Pomeroy, 1974; Azam et al., 1983; Sherr and Sherr, 1987) could convert suspended bacterial biomass into sinking particles. In the Cariaco Basin, diverse communities of flagellated and ciliated protists inhabit the lower chemocline and anoxic waters (Stoek et al., 2003; Taylor et al., 2006; Edgcomb et al., 2011) hence the tetrahymanol peak. Certain zooplankton inhabit or migrate into...
oxygen-depleted environments, such as the Arabian Sea and Chile OMZs (Gowing and Wishner, 1998; Turner, 2002; Wishner et al., 2008; Escribano et al., 2009), and although there are no data for the Cariaco Basin, mesozooplankton feeding within the chemocline can not be ruled out. Collisions between suspended particles and sinking detritus from surface waters that contain “sticky”

**Glycolipids**

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**Phospholipids**

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<td>X PDME-DAG</td>
<td><img src="image" alt="PDME-DAG" /></td>
<td>Phosphatidyl-(N,N)-dimethylethanolamine diacylglycerol</td>
</tr>
<tr>
<td>XI PE-DEG</td>
<td><img src="image" alt="PE-DEG" /></td>
<td>Phosphatidylethanolamine dietherglycerol</td>
</tr>
<tr>
<td>XII PME-DEG</td>
<td><img src="image" alt="PME-DEG" /></td>
<td>Phosphatidyl-(N)-methylethanolamine dietherglycerol</td>
</tr>
<tr>
<td>XIII lyso-DPG-DAG</td>
<td><img src="image" alt="lyso-DPG-DAG" /></td>
<td>Lyso Diphosphatidyl diacylglycerol</td>
</tr>
</tbody>
</table>

**Aminolipids**

<table>
<thead>
<tr>
<th>Type</th>
<th>Structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>XIV Betaine lipid DGTS</td>
<td><img src="image" alt="DGTS" /></td>
<td>Diacylglycerylhydroxymethyltrimethyl-(N,N,N)-homoserine</td>
</tr>
<tr>
<td>XV Betaine lipid DGTA</td>
<td><img src="image" alt="DGTA" /></td>
<td>Diacylglycerylhydroxymethyltrimethyl-(N,N,N)-β-alanine</td>
</tr>
</tbody>
</table>

**Archaeal Glycolipids**

<table>
<thead>
<tr>
<th>Type</th>
<th>Structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>XVII Gly-GDGT-0</td>
<td><img src="image" alt="Gly-GDGT-0" /></td>
<td>Monoglycosyl glycerol dibiphytanyl glycerol tetraether (acyclic)</td>
</tr>
<tr>
<td>XVIII 2Gly-crenarchaeol</td>
<td><img src="image" alt="2Gly-crenarchaeol" /></td>
<td>Diglycosyl crenarchaeol</td>
</tr>
<tr>
<td>XIX 2Gly-OH-GDGT-2</td>
<td><img src="image" alt="2Gly-OH-GDGT-2" /></td>
<td>Diglycosyl glycerol dibiphytanyl glycerol tetraether (with 2 rings)</td>
</tr>
</tbody>
</table>
organic coatings (e.g., transparent extracellular polymers; Passow, 2000; Passow et al., 2001) will enhance physical aggregation (Burd and Jackson, 2009) and vertical transport of otherwise suspended material. However, because Cariaco Basin trap material and sediments have not been thoroughly investigated for chemoautotrophic biomarkers, a strong link between water column chemoautotrophy and the sediment record has not been established. Diploptene in Cariaco sediments may derive from the water column (Freeman et al., 1994; Werne and Hollander, 2004; see also Elvert et al., 2001), but crenarchaeol in Cariaco Basin sediment trap material is thought to originate primarily from surface waters rather than at the chemocline (Turich and Wakeham, unpublished results; see also Wakeham et al., 2003 for the crenarchaeol in the Black Sea). Long-chain methoxy fatty acids from the suboxic zone were not detected in trap material and sediments (Wakeham et al., 2010).

On the other hand, there are examples of biomarkers derived from mid-water chemoautotrophy being present in sinking particles and recent sediments at other locations. In the Arabian Sea, material collected in sediment traps contain bacteriohopanepolyol (including BHP I), archaeal lipids (crenarchaeol) and ladderane lipids produced by chemoautotrophy in the OMZ (Wuchter et al., 2006; Jaeschke et al., 2007; Sáenz et al., 2011). BHP I in Recent Peru sediments is thought to originate in the suboxic zone of the overlying water column (Sáenz et al., 2011). The chemocline in the Black Sea is also a locus of chemoautotrophy (Wakeham et al., 2007), but unlike the Cariaco Basin, it often is within the photic zone. Thus in addition to chemoautotrophic biomarkers discussed above, SPM at the chemocline of the Black Sea is enriched in isorenieretene, a carotenoid derivative biosynthesized by anoxygenic photosynthetic green sulfur bacteria (Chlorobiaceae) (Repeta et al., 1989; Repeta and Simpson, 1991). Isorenieretene initially associated with SPM accumulates in Black Sea sediments and records vertical migrations of the chemocline into and out of the photic zone over the Holocene (Repeta, 1993; Sinninghe Damsté et al., 1993).

In conclusion, chemoautotrophy in marine water columns produces diagnostic biomarkers that provide valuable information about the biogeochemical processes at work. Many of the same biomarkers are widely used as molecular fossils to infer ancient oceanic environments, in particular during OAEs (e.g.,

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**Bacteriohopanepolyols**

- **XV BHT** Bacteriohopanetetrol
- **XVI** BHpentol
- **XVII** Aminotriol

**Ladderane FA**

- **XVIII** 18-[5]-ladderene FA
- **XIX** 20-[5]-ladderene FA
- **XX** 18-[3]-ladderene FA
- **XXI** 20-[3]-ladderene FA

**Fatty acids**

- **XXII** 16:1ω7c
- **XXIII** 16:1ω7t

- **XXIV** 9-CH₃O-16
- **XXV** 10-CH₃O-16

**Apolars**

- **XXVI** Diploptene
- **XXVII** Tetrahymanol
- **XXVIII** PMI

- **XXIX** Squalene
- **XXX** Lycopane

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**Fig. A2**
Koopmans et al., 1996; Kuypers et al., 2001; Hinrichs et al., 2003; Brooks and Pearson, 2005). Such hind-casting interpretations require a sound understanding of biogeochemical processes in the contemporary ocean. Further research in the Cariaco Basin and similar environments will be needed to fully confirm relationships between water column chemosuotrophy and its manifestation in the sediment record.

Acknowledgments


References


Elvert, M., Suess, E., 1999. Anaerobic methane oxidation associated with hydrothermal vents at Omega and similar environments will be needed to fully confirm relationships between water column chemosuotrophy and its manifestation in the sediment record.

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