Compound-specific $\delta^{15}\text{N}$ and chlorin preservation in surface sediments of the Peru Margin with implication for ancient bulk $\delta^{15}\text{N}$ records.

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Abstract

Understanding the processes that control the preservation of paleoceanographic proxies is of clear importance. Surface sediments from the Peru Margin oxygen-minimum zone are subject to lateral and downslope transport by bottom currents that decrease organic matter (OM) quality. Indicators of bulk OM quality (pyrolysis hydrogen index, pyrolysis S1 + S2 and C/N) demonstrate significant degradation between 150 and 400 m water depth, within the oxygen-minimum zone. Concentrations of the three most abundant chlorins (chlorophyllone, pheophytin and pyropheophytin) decrease from 750 to 150 nmoles*gTOC⁻¹ from 150 to 400 meters water depth though the relative abundances of the chlorins in an individual sample do not change. This suggests that the three chlorins have similar reactivity over the ambient conditions. Values for $\delta^{15}\text{N}$ of bulk sediments ($\delta^{15}\text{N}_{\text{bulk}}$) decrease by 3‰ from the inner shelf to the upper slope (1000 m) but co-occurring compound-specific $\delta^{15}\text{N}$ values ($\delta^{15}\text{N}_{\text{chlorin}}$) do not decrease downslope. The low variability of $\delta^{15}\text{N}_{\text{chlorin}}$ values supports a single source for the chlorins, and demonstrates the recalcitrance of $\delta^{15}\text{N}_{\text{chlorin}}$ values despite degradation. This set of observation raises questions about which type of OM fraction best records ‘primary’ signatures. We assess two possible models to guide our interpretation of these disparate datasets (1) that decreasing $\delta^{15}\text{N}_{\text{bulk}}$ values are the result of degradation of a ¹⁵N-enriched fraction during downslope transport, and that $\delta^{15}\text{N}_{\text{chlorin}}$ values reflect primary values; (2) that $\delta^{15}\text{N}_{\text{bulk}}$ values are primary and that chlorins are derived from material transported from upslope. These data reaffirm that in active sedimentary environments such as the Eastern Tropical Pacific, transport of OM can significantly alter bulk geochemical parameters of OM integrity, but the impacts on the $\delta^{15}\text{N}$ record of bulk sediments and chlorins are less clear, and require more study to be thoroughly understood.

1 Introduction

Characterizing the preservation of geochemical proxies in modern environments is extremely important as we apply novel parameters to ancient environments. The geochemical records preserved in sedimentary sequences of the Peru Margin and in other parts of the Eastern Tropical Pacific have been used to characterize and quantify paleoceanographic changes associated glacial-interglacial transitions (Altabet et al., 1995; Ganeshram et al., 2000; Kienast et al., 2002; Galbraith and Kienast, 2013) and are used to guide interpretations in ancient analog nitrogen cycle studies (e.g. Algeo et al., 2008). Many of these studies also rely upon nitrogen isotopes and concentrations of chlorophyll derivatives (chlorins) as paleoproductivity indicators (Liu et al., 2008; Higginson et al., 2003), and are integral to our understanding of the impacts of rapid climate change on ocean ventilation.

Arthur et al. (1998) demonstrated that OM in surface sediments from the Peru Margin OMZ was less well preserved than would be expected despite low dissolved oxygen concentrations ($<5 \mu\text{mol/kg}$) where the OMZ impinges on the seafloor. Poor preservation was attributed to advection of low concentrations of dissolved oxygen, activity of low-oxygen tolerant organisms, and particularly, resuspension and lateral transport of OM by strong bottom currents at the shelf break. Nepheloid transport of OM is a process that has been recognized in other localities such as the Benguela Upwelling system of offshore Namibia and elsewhere (Ohkouchi et al., 2002; Mollenhauer et al., 2005; Mollenhauer et al., 2007). Bottom currents increase the residence time of sediments at the sediment water interface extending exposure to oxidants (dissolved oxygen, nitrate, iron oxides and sulfate) and allowing progressive degradation that can negatively impact the fidelity of paleoceanographic proxies.

The preservation of primary $\delta^{15}\text{N}$ signals in ancient sediments has been an issue of discussion and concern for studies utilizing $\delta^{15}\text{N}$ data (Altabet et al., 1999; Sachs and Repeta, 1999; Junium and Arthur, 2007; Robinson et al., 2012) and is the basis for interest in compound-specific $\delta^{15}\text{N}$ analyses (Sachs and Repeta, 2000; Ohkouchi et al., 2006; Kashiyaama et al., 2008; Kusch et al., 2010; Higgins et al., 2012). Enrichment of $\delta^{15}\text{N}$ values of sinking OM in oxic water-columns has been well characterized (Altabet, 1988; Freudenthal et al., 2001); however in low-oxygen settings water-column diagenetic

alteration is generally assumed to have a negligible effect on $\delta^{15}\text{N}$ values. No studies have focused specifically on how nitrogen isotope values vary under the range of conditions that are present in core-top sediments down-slope in a modern upwelling zone. Previous research on Peru Margin sediments and similar margins provides an excellent background for determination of the influence of transport on $\delta^{15}\text{N}$, and the preservation of chlorophyll *a*-derived chlorins used in compound specific $\delta^{15}\text{N}$ studies.

Here we present $\delta^{15}\text{N}$ data from bulk sediments and photosynthetic pigments, as well as chlorin concentrations from core top sediments of the Peru Margin from two depth transects that intersect the (OMZ). Our goal is to document the impact of transport on the preservation of $\delta^{15}\text{N}$ and chlorophyll derivatives. These results have implications for bulk nitrogen isotope and pigment paleo-data in modern and recent upwelling zones.

2 Materials and Methods

2.1 Material Collection and Preparation.

Samples were recovered with submersible executed push cores and ship-deployed box cores on two transects at 12° and 13.5° South latitude during December-January of 1991 and 1992 (Fig. 1). Retrieved box cores and push cores were sub-sampled and refrigerated or frozen shipboard. Samples for bulk analysis were freeze-dried, homogenized and archived in Sedimentary Geology Lab in The Pennsylvania State University Department of Geosciences, until analysis. Sub-samples for compound-specific $\delta^{15}\text{N}$ were taken from samples that have been frozen since sampling. Sampling was focused on the inner-shelf (~100 m) through the upper-slope to 1070 m. From the inner-shelf to the uppermost slope the water column is largely anoxic and the oxygen-minimum zone (OMZ) impinges upon the bottom (Fig. 1). Below ~600 m in both transects oxygen concentrations rise, and reached ~50 $\mu\text{mol}\cdot\text{kg}^{-1}$ by 1000 m at the time of sampling. Sediment characteristics are generally controlled by the presence of bottom currents and the degree of phosphogenesis (Arthur et al., 1998). Inner-slope settings are dominated by mud surfaces with common *Thioploca* microbial mats (Høgslund et al., 2009). As current velocities increase at the shelf-break, megarippled mud-surfaces, phosphorite crusts and sands predominate. Below 600 meters, current velocities decrease and sediments cover is dominated by mud and glauconite sands.

2.2 Bulk Carbon and Nitrogen Analyses

Freeze-dried samples were treated at room temperature for 24 hours with buffered acetic acid (pH 4) to remove carbonate minerals and to preclude hydrolysis of proteinaceous OM. Decarbonated residues were rinsed to remove residual acid. Isotopic analyses for nitrogen were performed using a Costech/Thermo-Finnigan Delta Plus XP, coupled elemental analyzer, continuous flow, isotope-ratio mass spectrometer (EA-CF-IRMS). All analyses were performed in the Stable Isotope Biogeochemistry Lab at The Pennsylvania State University. Powdered, decarbonated samples were weighed and sealed in tin boats for isotopic analysis. Samples were combusted at 1020°C with a “zero blank” helium atmosphere autosampler that has been retrofitted to include a custom vacuum purging system. Data are reported using delta notation relative to atmospheric N₂ for nitrogen. Reference gases were calibrated relative to standards IAEA N1 and N2 in combination with in-house, Devonian black shale and Peru mud isotopic standards. Standard precision was often better than ±0.15‰ for N but is reported as ±0.2‰ to reflect reported precision from known isotopic values of IAEA nitrogen standards. Calibration of samples to reference data was performed using the multi-point calibration method described in Coplen et al. (2006). Carbon and nitrogen percentages on the decarbonated fraction were produced on a Costech Elemental analyzer in conjunction with isotopic measurements. Calibrations were achieved using acetanilide (Costech), Devonian black shale and Peru Mud in-house standards of known elemental composition with a standard error of ± 0.1% for carbon and ± 0.1% for nitrogen. C/N values are reported as atomic ratios.

2.3 Rock Eval Pyrolysis

Rock-Eval pyrolysis data were produced using a RockEval II instrument and TOC data were produced using a UIC Coulometrics Total Carbon Analyzer, the methods for which are reported in Arthur et al., (1998). Rock-Eval pyrolysis is a tool for assessing the type of organic matter, its hydrocarbon richness (controlled by preservation and quality) and its thermal maturity. In the case of recent surface sediments thermal maturity is low, and changes in Rock-Eval parameters are associated with the relative preservation of organic matter during early diagenetic degradation. We report hydrogen indices (HI), S1 and S2 data. Rock-Eval pyrolysis heats a sample progressively and measures the quantity

of pyrolyzable material as measured by a flame-ionization detector. S1 and S2 are the first two peaks observed during pyrolysis. The S1 peak contains the ‘free’ hydrocarbons released at a constant temperature of 350°C that in thermally mature sediments would constitute the oil and gas phases in a source rock. In immature sediments the S1 peak constitutes the free organic compounds that are not yet incorporated into protokerogen. The S2 peak contains the hydrocarbons that are released from sample powders during progressive heating of the sample to 550°C and comprise the thermal cracking of kerogens and protokerogens. S1 and S2 values are the mg of hydrocarbon per gram of dry sediment. Hydrogen indices (HI) are S2 values normalized to the quantity of organic carbon. Oxygen indices (OI) are typically reported with HI and are the quantity of CO₂ generated during pyrolysis and reflect the quantity of oxygen in organic matter or in calcium carbonate. High oxygen indices can be found in samples where organic maturity is very low, heavily oxidized or where carbonate is present. OI values in the Peru Margin sediments are high (~200) because of the presence of carbonate (Supplementary material). Here we report S1 + S2 data. This is not a typical treatment of pyrolysis data but given that these are recent surface sediments the free hydrocarbon fraction (S1) and the protokerogen fraction (S2) likely remain available during early diagenetic cycling of organic matter.

2.4 Pigment Analyses

Freeze-dried sediments were sonically extracted in acetone to clarity under low-light and in a cold water bath. Extracts were filtered through a plug of solvent extracted cotton wool, evaporated to dryness under N₂-atmosphere and frozen until time of analysis. Reverse phase HPLC analysis of total acetone extracts was conducted in the Biogeochemistry Labs at The Pennsylvania State University, using an Agilent 1200 HPLC system equipped with an eight channel multi-wavelength detector. Separations were achieved using two Waters Spherisorb ODS2 columns (4.6 mm x 150 mm; 3 µm) linked in series with a Phenomonex, Security Guard cartridge pre-column assembly. Aliquots of acetone extracts were analyzed using a quaternary gradient elution program comprising vacuum degassed acetonitrile, methanol, water and ethyl acetate over 85 min with a flow rate of 0.7 ml*min⁻¹ (cf. Airs et al., 2001). LC-MSⁿ analysis was performed using an Agilent 3150 ion trap mass spectrometer with an atmospheric pressure chemical

ionization source. Chlorin quantification was achieved via on-line UV-Vis absorbance data (665 μ m). Calibrations were performed with known quantities of a pyropheophytin standard and normalized for solvent composition at time of elution and reported in nanomoles*gTOC⁻¹.

2.5 Compound-specific $\delta^{15}\text{N}$ analyses of pigments

A limited number of large volume samples were available (6) that had been frozen since the time of sampling in 1991. This allowed for a limited number of compound-specific isotope analyses of the three most abundant pigments (chlorophyllone, pheophytin and pyropheophytin) from 130 to 989m water-depth in these samples (Fig. 2). The 2-dimension HPLC purification employed in this study is the preferred method to purify pigments for isotopic analysis (Sachs and Repeta, 2000). Base line resolution between peaks in the normal phase purification step (detailed below) is necessary to assure compound purity (cf. Kashiya et al., 2007) and was achieved for all samples analyzed.

Samples were fraction collected from quaternary gradient reversed phase effluent (Fig. 2) and dried under N₂ stream and frozen until further purification. Normal phase purifications for pheophytin and pyropheophytin were adapted from Sachs and Repeta (2000) and utilize an isocratic solvent composition of 8% acetone in hexane at 2ml*min⁻¹ on two, 25cm Agilent 5 μ m Sil analytical columns linked in series. Concentrated samples were dissolved in 8% acetone in hexane and injected in 20-40 μ l aliquots, dependent on pigments concentration. Under isocratic solvent elution, pheophytin displays significant peak tailing but it is overcome by utilization of a mild gradient elution that increases acetone concentrations to 20% after 4 minutes. It should be noted that the isocratic solvent composition and gradient needs to be adjusted in accordance with the brand of column, size of the stationary phase, overall column condition and presence of potential co-eluting compounds. Purification of chlorophyllone is not possible on a silica column as it partitions too strongly onto the silica stationary phase; elution is possible under high concentrations of acetone, but the resulting peak is overly broad and suffers from significant coelution.

Purification of chlorophyllone for isotopic analysis has not been reported previously and was not possible using established methods for the pheophytins and

pheophorbides. Baseline resolution separation was achieved using a 20% acetone in hexane solution on a 15cm Restek 5 μ m amino column under isocratic conditions (Fig. 3). Additional checks for compound purity included assessments of chlorin quantity determined from online UV/Vis data with respect to the quantity of nitrogen in the combusted sample and the C/N ratio. If samples with C/N ratios that were in consistent with pure chlorins they would not be considered (cf. Sachs and Repeta, 2000). We did not acquire sufficient sample quantities for all three chlorins in all samples and the lack of a particular a chlorin in a sample reflects this fact (Fig. 5).

Isotopic analyses of chlorins were conducted using a modified elemental analysis, isotope ratio mass spectrometer (EA-IRMS) system that employs a cyro-trapping/capillary-column focusing method. Details of this method, the analytical system and its capabilities are detailed in Polissar et al., (2008). All data are reported using standard, delta notation and calibrated within individual runs to octaethylporphyrin (Frontier Scientific), amino acids (methionine and alanine) in-house standards and IAEA N1, N2 and run with procedural blanks.

3 Results

3.1 TOC and Rock Eval Pyrolysis

Total organic carbon (TOC), and pyrolysis data were originally reported in Arthur et al. (1998). To benefit presentation and analysis we merged data from the two depth transects. Arthur et al., (1998) demonstrated that similar patterns in the distribution of TOC and sediment type exist between the two transects, suggesting that OM sources are very similar and justifies merging the two sample sets. TOC and HI values are generally highest on the anoxic regions of the anoxic shelf and but decrease in the anoxic upper slope (Fig. 4). Outlier, low TOC values from the inner-shelf are associated with *Thioploca* microbial mats (Fig. 4), and elevated TOC and pyrolysis values below the OMZ may represent rapidly redeposited material from up-slope though these samples only have high TOC values (Fig. 4) (Arthur et al., 1998). RockEval S1 and S2 data, defined as milligrams of hydrocarbons per gram dry weight (mgHC*g⁻¹) released during heating and thermal cracking of (proto)kerogens. They are useful for comparison to other forms of data such as C/N as they are not calculated using %TOC (unlike HI), and for

this reason we rely primarily on S1+S2, rather than HI for comparison to other geochemical parameters. S1+S2 values vary significantly over the upper 400 m, but below 200 m S2 values decrease to values below 5 mgHC*g⁻¹. Oxygen indices are uniformly high and are reflective of the minor, but consequential concentrations of calcium carbonate.

3.2 C/N Ratios

In sediments where the OM is of marine origin, variability in C/N ratios can be useful as an indicator of differential preservation of C and N pools (Junium and Arthur, 2007; Freudenthal et al., 2001). C/N ratios of phytoplankton biomass typically range from 4 to as high as 10, and degradation under most conditions results in higher C/N ratios resulting from degradation of low C/N biomass such as proteins and nucleic acids (e.g. Macko et al., 1986). Alternatively, if degrading phototrophic biomass is replaced by heterotrophic bacterial OM, C/N ratios can remain low (Lehmann et al., 2002). Additional factors such as sorption of ammonium onto clays in sediments can also act to depress C/N ratios low as OM degrades (e.g. Peters, 1978). Peru margin shelf C/N values remain relatively low (average of 11) in the anoxic shelf region and increase slightly in the upper region of the Anoxic Upper Slope, but are more variable downslope (Fig. 4).

3.3 Bulk $\delta^{15}\text{N}$

Shelf and upper-slope $\delta^{15}\text{N}_{\text{bulk}}$ values range between +6.5 and +10.9‰ and average +8‰. At 350 m water depth $\delta^{15}\text{N}_{\text{bulk}}$ values decrease to less than +6‰ below 700 m (Fig. 4). The observed $\delta^{15}\text{N}_{\text{bulk}}$ values are typical of surface sediments and particulates from upwelling zones (Altabet et al., 1999).

3.4 Geochemical Parameters Relationships

For S1 + S2 values below 20 mgHC*g⁻¹ we observe more scatter in $\delta^{15}\text{N}_{\text{bulk}}$ and C/N (Fig. 5). Where S1 + S2 values are greater than 20 mgHC*g⁻¹, C/N ratios range more narrowly between 12 and 9; for S2 values below 20 mgHC*g⁻¹, C/N values range from 10 to 30 and increase as S1 + S2 values decrease. $\delta^{15}\text{N}_{\text{bulk}}$ values display relationships with %N, HI, and S1 + S2 though no significant relationship with C/N. The trend between %N and $\delta^{15}\text{N}_{\text{bulk}}$ is very similar in appearance to the relationship between S2 and $\delta^{15}\text{N}$ (Fig. 5). $\delta^{15}\text{N}_{\text{bulk}}$ values increase slightly as %N drops from 3% to 1% and decrease below 1% N. When % N is less than 0.7%, all $\delta^{15}\text{N}_{\text{bulk}}$ values are less than

6.7‰; a similar trend is observed for HI vs. $\delta^{15}\text{N}_{\text{bulk}}$, where most $\delta^{15}\text{N}_{\text{bulk}}$ values that are lower than 6.7‰ have corresponding HI values that are below 225. A general trend of increasing C/N with increasing $\delta^{15}\text{N}_{\text{bulk}}$ is also observed (Fig. 5); however, the relationship is not statistically significant (Fig. 5). Relationships between C/N and $\delta^{15}\text{N}_{\text{bulk}}$ have been observed in other organic-matter rich units such as Cretaceous black shales (Junium and Arthur, 2007) or Mediterranean Sapropels (Higgins et al., 2010). However, in Cretaceous examples, elevated C/N values correspond to better OM preservation, indicated by a positive relationship between HI and C/N (Junium and Arthur, 2007).

3-5 Chlorins

HPLC-UV/Vis and LC-MSⁿ data of acetone extracts reveal six functionalized chlorins that are degradation products of chlorophyll *a* and are the most abundant pigments in the Peru Margin surface sediments (Figs. 2 and 4). All chlorins were identified on the basis of strong absorbances at 655nm, elution order and MSⁿ mass spectra (Airs et al., 2001). Two closely eluting peaks at 11 and 12 minutes (Fig. 2, peaks 1 and 2) display full mass spectra that are dominated by single ions at *m/z* 533 are consistent with protonated masses [*M*/*H*⁺] of chlorophyllone epimers (Harris et al., 1995; Mawson and Keely, 2008). Chlorophyllone is a common constituent of sedimentary core-tops and water-column particulates and is formed from the cyclization of the chlorophyll *a* propionic chain at C17 with C13² as a result of detoxification during heterotrophy (Kashiyama et al., 2012; Kashiyama et al., 2013). Two small peaks eluting at 43 and 44 minutes (Fig. 2, peaks 3 and 4) have full mass spectra that are dominated by a single ion at *m/z* 887 and an MS² spectra indicating loss of the esterifying alcohol phytol, and are consistent with protonated masses [*M*/*H*⁺] of hydroxychlorophyll *a* epimers (Stuart Walker and Keely, 2004).

Peak 5 (Fig. 2) has a full mass spectra displaying a single ion at *m/z* 871 and an MS² spectra consistent with the loss of phytol and is consistent with protonated masses [*M*/*H*⁺] of pheophytin *a*. Peak 7 (Fig. 2) has a full mass spectra at *m/z* 814 and an MS² spectra indicating the loss of phytol and is consistent with protonated masses [*M*/*H*⁺] of pyropheophytin *a*. No Mg-chlorophylls, phaeophorbide or non-chlorophyll *a*

tetrapyrroles, such as chlorophyll *c* were found in quantities sufficient for identification. Minor peaks are consistent with a variety of carotenoids, chlorins and late eluting steryl-chlorin esters.

Chlorophyllone is the most abundant chlorin in all samples with an average chlorophyllone/(pheophytin + pyropheophytin) ratio of 3.7 +/-1.5. The most abundant chlorin concentrations are present at the inner slope sites (Fig. 4). Chlorin concentrations decrease with increasing water-depth, decrease sharply at slope break.

3-6 Chlorin $\delta^{15}\text{N}$

$\delta^{15}\text{N}_{\text{chlorin}}$ values for chlorophyllone, pheophytin and pyropheophytin average 0.2‰ and range between -1 and +1.6‰ (Fig. 6), whereas corresponding $\delta^{15}\text{N}_{\text{bulk}}$ values range from +5.4‰ to +10.9‰ (Fig. 6). Chlorin isotopic compositions display a strong correspondence between the different structures in individual samples and show no trend with water depth or distance offshore (Fig. 6). The offset between $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{chlorin}}$ is typical of chlorophyll derivatives and is the result of biochemical fractionation during chlorophyll biosynthesis (Sachs and Repeta, 2000). The $\Delta^{15}\text{N}_{\text{bulk-chlorin}}$ for Peru Margin sediments averages +7‰. The range of $\Delta^{15}\text{N}_{\text{bulk-chlorin}}$ (referred to as ϵ_{por}) values from marine eukaryotic phytoplankton grown in culture and from ancient sedimentary sequences where eukaryotes were dominant average average ~+5‰ and range between +3‰ and +8‰ (Sachs and Repeta, 2000; Higgins et al., 2011; Tsao et al., 2012; Higgins et al., 2012). Our ϵ_{por} values for the Peru Margin surface sediments are higher than is typical, and $\delta^{15}\text{N}_{\text{bulk}}$ values have a greater range than $\delta^{15}\text{N}_{\text{chlorin}}$.

4 Discussion

4-1 Organic Matter Transport

Dynamic depositional environments, such as the Peru Margin continental slope, can increase the residence time of organic matter at the sediment water interface exposing organic matter to prolonged degradation even in low-oxygen conditions. On the basis of hydrocarbon richness (S1 + S2 and HI) values, C/N ratios, TOC (wt. %) it is clear that OM degrades progressively downslope (Fig. 4). The steepest decreases in preservation parameters occur from 200 to 400 meters water depth in the core of the oxygen-minimum zone. Arthur et al. (1998) attributed the decrease over that depth range to the action of

benthic currents that advect low concentrations of dissolved oxygen ($<5 \mu\text{mol/kg}$), the activity of benthic organisms, and lateral transport of OM by strong bottom currents up to 30 cm/s that initiate at the shelf break.

Lateral, downslope and nepheloid transport of sediment and organic matter has been detailed in many marine environments (Arthur et al., 1998; Ganeshram et al., 1999; Levin et al., 2002; Inthorn et al., 2006; Mollenhauer et al., 2007). It is clear that transport results in anomalous age distributions in various organic reservoirs in surface sediments (Ohkouchi et al., 2002; Mollenhauer et al., 2005). Mollenhauer et al. (2007) demonstrate that resuspension and transport results in age increases of up to 2000 years in the age of bulk sediments and compound-specific fractions from the shelf to the upper slope in the Benguela Upwelling system off the coast of Namibia. Limited radiocarbon age data from the Peru Margin at 12°S demonstrate that ages increase significantly as water depth and transport distance increase. Surficial sediments have radiocarbon ages of 620 years at 133 m water depth and 1210 years at 480 m water depth (Burnett et al., 1988).

Extension of the residence time of OM at or above the sediment water interface allows for progressive degradation of organic matter that may impact geochemical proxy data. In the case of the Peru Margin sites studied here, we suggest that degradation is the result of extended exposure to oxidant in a manner similar to that described by Hartnett et al., (1998) (Hartnett et al., 1998) and Hedges and Keil, (1995) (Hedges and Keil, 1995) . Here the primary oxidant is not dissolved oxygen, but NO_3^- (Van Mooy et al., 2002), FeOOH (Froelich et al., 1988) and SO_4^{2-} (Canfield et al., 2010) mediated by anaerobic metabolism in the heart of the anoxic Peru Margin OMZ. Additional factors such as winnowing and hydrodynamic sorting of size fractions and types of OM during transport increase heterogeneity and could further affect proxy data (Bergamaschi et al., 1997). Regardless of the relative role of sorting or exposure time, it is clear that extended time near the sediment water interface results in greater OM degradation and increased potential for alteration of paleoceanographic proxies.

4.2 $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{chlorin}}$

Many studies have utilized $\delta^{15}\text{N}_{\text{bulk}}$ data from low-oxygen upwelling environments such as the Eastern Tropical Pacific or Arabian Sea (Altabet et al., 1995;

Ganeshram et al., 1995; Altabet et al., 1999; Ganeshram et al., 2000; Agnihotri et al., 2008) to reconstruct variability in the nitrogen cycle over glacial-interglacial cycles. The majority of these studies observe similar signals supporting the efficacy of this approach (Galbraith and Kienast, 2013). This has ultimately led to the use of $\delta^{15}\text{N}_{\text{bulk}}$ in quantifying and confirming N-cycle models that provide benchmarks for understanding glacial to interglacial transitions, inform future deoxygenation scenarios and related N-Cycle impacts (Meissner et al., 2005; Schmittner and Galbraith, 2008; Keeling et al., 2010).

In light of the role of transport on the degradation organic matter in the Peru margin sediments (Arthur et al., 1998; Levin et al., 2002) we aimed to test the resistance of $\delta^{15}\text{N}_{\text{bulk}}$. We examined the relationship between $\delta^{15}\text{N}_{\text{bulk}}$, bulk measures of organic matter quality (HI, S1 + S2, C/N) (Fig. 5) and $\delta^{15}\text{N}_{\text{chlorin}}$ from selected samples. As OM quality decreases downslope we observe variability and ultimately lower $\delta^{15}\text{N}_{\text{bulk}}$ values (Figs 4 and 5). Diagenetically resistant $\delta^{15}\text{N}_{\text{chlorin}}$ values do not display the same range (Fig. 6) and suggests that $\delta^{15}\text{N}$ variability in high-productivity, low-[O₂] environments may be impacted by diagenesis or other factors such as growth of benthic microbial biomass. A diagenetic control on $\delta^{15}\text{N}_{\text{bulk}}$ is contrary to our understanding that post-depositional changes in $\delta^{15}\text{N}_{\text{bulk}}$ values are negligible, or that diagenetic reactions typically result in ¹⁵N-enrichment (e.g. Robinson et al., 2012). Early diagenetic ¹⁵N-enrichment is well described in sinking particles and sediments under oxidizing conditions during microbial degradation (Altabet and Francois et al., 1994; Freudenthal et al., 2001; (Gaye-Haake et al., 2005) but sinking particles in anoxic conditions do not display the significant $\delta^{15}\text{N}$ differences (Altabet et al., 1999). If the net result of degradation downslope was to lower $\delta^{15}\text{N}_{\text{bulk}}$ values, this is not typical of our understanding of diagenetic influences on organic nitrogen.

For the purposes of discussing the range of possibilities that may explain the disparity between $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{chlorin}}$ we consider two opposing scenarios for Peru Margin surface sediments: **(1)** that $\delta^{15}\text{N}_{\text{bulk}}$ values are subject to change during the degradation of OM during transport, and that $\delta^{15}\text{N}_{\text{chlorin}}$ retains primary nitrogen cycle signals; and the alternative **(2)** that decreases in $\delta^{15}\text{N}_{\text{bulk}}$ values reflect primary nitrogen cycle changes, and that chlorins are transported from a single source on the shelf.

To assess the $\delta^{15}\text{N}_{\text{bulk}}$ record we follow the simple but robust assumption that $\delta^{15}\text{N}_{\text{chlorin}}$ values have not been altered by secondary processes. This is reasonable because chemical attack on the chlorin macrocycle occurs at the methine bridges between pyrrole units. If this occurs, the chlorin is destroyed. Additionally, the chlorin nitrogens are shielded from the methine carbon by a pyrrole carbon and unlikely to express any nitrogen isotope effects during breaking of the bonds with any of the alkyl substituents or functional groups that are bonded to the tetrapyrrole structure. The three chlorins present in quantities sufficient for compound-specific $\delta^{15}\text{N}$ have nearly equivalent values for $\delta^{15}\text{N}_{\text{chlorin}}$ further supporting recalcitrance for the chlorin-N and a common chlorophyll *a* precursor.

The relatively small variability in $\delta^{15}\text{N}_{\text{chlorin}}$ and changing values for ϵ_{por} suggest that $\delta^{15}\text{N}_{\text{bulk}}$ values changed relative to $\delta^{15}\text{N}_{\text{chlorin}}$. However, the mechanism for the changes in $\delta^{15}\text{N}_{\text{bulk}}$ is not entirely clear. Any one sample is a mixture of organic matter derived from transported sediment from the shelf break and organic material sinking from the water column rapidly as fecal pellets or more slowly as marine snow. Discerning the range of processes that may have acted on any particular package of material becomes difficult because of the mixing of signals. Sinking of organic material through the water column and benthic downslope transport ultimately had the same effect, extension of the residence time in an area where organic matter can be easily degraded. We speculate that degradation of organic matter can result in two $\delta^{15}\text{N}_{\text{bulk}}$ trajectories, one that is the result of the standard ^{15}N -enrichment as a result of kinetic isotope effects (Macko and Estep, 1984; Macko et al., 1993) and the second that results in ^{15}N -depletion through the complete degradation of a more reactive fraction of ^{15}N -enriched organic matter.

One possible mechanism for the shift to lower $\delta^{15}\text{N}_{\text{bulk}}$ and ϵ_{por} values below 500 is the selective degradation of a labile ^{15}N -enriched OM fraction such as amino acids and proteins (Prokopenko et al., 2006; Tesdal et al., 2013). Amino acid nitrogen is the most important source of inorganic nitrogen in the water column and sediments of modern anoxic basins (Burdige and Martens, 1988; Pantoja and Lee, 2003). In OMZ environments, such as that of Peru, proteins and amino acids are targeted by heterotrophic denitrifying bacteria as a carbon source, resulting in dissimilatory loss of N (Van Mooy et al., 2002). The total N (wt. %) as amino acids (%T_{AA}N) in surface

sediments of upwelling zones can be as high as 70% of the sedimentary N fraction, though values are typically lower (Pantoja and Lee, 2003). Lomstein et al., (2006) (Lomstein et al., 2006) demonstrate that in Peru Margin surface sediments %T_{AA}N values are nearly 50% at shallow sites but decrease to less than 20% below 800 m. In general, amino acid nitrogen is ¹⁵N-enriched relative to bulk biomass (Macko et al., 1986) but is variable dependent on the specific amino acid (McClelland and Montoya, 2002). Tesdal et al., (2013) suggest a model where initial ¹⁵N-enrichment is the result of degradation of ¹⁵N-depleted amino acids and preservation of ¹⁵N-enriched amino acids such as glutamate (McClelland and Montoya, 2002) which are found in diagenetically resistant cell walls (Pedersen et al., 2001). Ultimately, the less reactive amino acid N-fraction is degraded resulting in ¹⁵N-depletion. Our data support this hypothesis; $\delta^{15}\text{N}_{\text{bulk}}$ values increase 1‰ to 2‰ between 200 m and 400 m in the region of fastest currents and largest decreases in S1 + S2, and transition to lower $\delta^{15}\text{N}_{\text{bulk}}$ values where mildly oxidizing conditions return below 600 m.

A possible source of the resistant, low-reactivity N may be a minor component of terrestrial organic matter, but carbon isotope and Rock Eval pyrolysis data are not consistent with a significant nonmarine component in the studied samples (Arthur et al., 1998). Additional factors contributing to ¹⁵N-depletion may include sorption of ammonium or nitrogenous organic matter to mineral surfaces. Degradation of organic N initially releases the ¹⁵N-depleted ammonium that fills available sites on mineral surfaces. As OM degradation continues the ammonium becomes progressively more ¹⁵N-enriched but is not retained because mineral surfaces have reached their sorptive capacity early in the diagenetic process and retain the initially ¹⁵N-depleted signature (Freudenthal et al., 2001). If ultimately a majority of the N in degraded sediments remains as ammonium-N, the $\delta^{15}\text{N}$ values are expected to be lower than primary organic-N (Rau et al., 1987).

Potential overprints on $\delta^{15}\text{N}_{\text{bulk}}$ values are also possible where the addition of benthic microbial biomass was significant. Two ¹⁵N-enriched samples from the inner-shelf stand out in our dataset (marked by boxes in Figs. 4 and 5) and are associated with significant contributions from *Thioploca* biomass evident as white microbial filaments in uncrushed samples, and seafloor observations from submersible. In these particular samples, TOC content is relatively low, but the *in situ* production of organic matter by

benthic microbial communities has resulted in anomalously high HI values. The elevated $\delta^{15}\text{N}$ values we observe are likely caused by fractionation during reduction of nitrate by *Thioploca* communities. *Thioploca* form dense mats over large areas of the Peru and Chile OMZ at around 200-400 m water depth and are present below the sediment water interface as well (Levin et al., 2002; Høglund et al., 2009). If *Thioploca* biomass were a significant contributor to ancient OM, their signature is capable of swamping primary $\delta^{15}\text{N}$ signals as we observe clearly in two samples from ~190 m.

The alternative scenario is that $\delta^{15}\text{N}_{\text{bulk}}$ values reflect changes in nutrient status from the overlying water column. Though we cannot discount an important role for a pelagic organic component, it seems likely that organic phases at deeper depths are principally derived from upslope. Sedimentary fabrics are consistent with a predominant role for transport of material as opposed to pelagic sedimentation for depths below 600 m (Levin et al., 2002) where $\delta^{15}\text{N}$ values are lowest. This leaves the possibility that the decrease in $\delta^{15}\text{N}_{\text{bulk}}$ is the result of the preservation of more ancient nitrogen cycle signals. Nitrogen isotope values from a core at 11.3 °S and 186 m water depth decrease from +7.5‰ to +5.5‰ from 350 to 500 years ago and remain relatively constant near 6‰ over the remaining 2000 years of the record (Agnihotri et al., 2008). These values are within the range of $\delta^{15}\text{N}_{\text{bulk}}$ values we report (Fig. 4), and it is possible that the decrease downslope represents preservation of more ancient nitrogen cycle signals. A water column signal or a more ancient signal suggests that pigments were derived from a source with a relatively consistent $\delta^{15}\text{N}$ such as the shelf, or reflect complex changes in phototrophic ecology inferred from decreasing ϵ_{por} values at greater depths (Fig. 6).

4.3 Chlorin Distribution and Downslope Trends

In all samples, chlorophyllone is present as two enantiomers (Fig. 2, Peaks 1 and 2), and is the most abundant chlorin, with pyropheophytin and pheophytin following in abundance (Fig. 4). Bicyclo-chlorins, including chlorophyllone and cyclopheophorbide-*a*-enol are commonly found in water column particulates, (e.g. Stuart Walker and Keely, 2004), core-top sediments (Chillier et al., 1993; Harris et al., 1995; Ocampo et al., 1999) and in more ancient sediments (Mawson and Keely, 2008; Junium and Arthur, 2007; Kashiya et al., 2008) and are the precursors of bicycloalknoporphyryns (BiCAPs)

(Junium et al., 2011). Recently, the recognition of high concentrations of 13^2 - 17^3 -cyclopheophorbide a-enol, the probable precursor for chlorophyllones, in heterotrophic protistan biomass suggests that herbivory is the primary pathway for formation of chlorophyllones in the geologic record (Kashiyama et al., 2012; Kashiyama et al., 2013). The Kashiyama et al., (2012) finding confirms previous suggestions that linked chlorophyllones with herbivory (Karuso et al., 1986; Goericke et al., 2000; Stuart Walker and Keely, 2004). Indeed, fecal pellets and marine snow are the primary delivery mechanisms for OM to the sediment water interface on the Peru Margin (Arthur et al., 1998) and most upwelling zones (Staresinic et al., 1983). The high ratio of chlorophyllone to pheophytin and pyropheophytin (3.7 ± 1.5) in Peru surface sediments suggests that at a high proportion of the phototrophic biomass in sediments was consumed by protistan herbivores and that chlorophyllones were derived from 13^2 - 17^3 -cyclopheophorbide a-enols. The asymmetry in abundance of the two chlorophyllone peaks is thought to be a result of enzymatic processes associated with herbivory (Aydin et al., 2003); this asymmetry is typical of chlorophyllones in modern and geologic examples (Junium et al., 2011; Mawson et al., 2007). Additionally, the similarity between the pheophytins and chlorophyllone $\delta^{15}\text{N}_{\text{chlorin}}$ lends further support to a common chlorophyll source, presumably chlorophyll *a*, for chlorophyllone.

Relative abundances of chlorophyllone, pheophytin and pyropheophytin remain surprisingly constant down-slope. It might be expected that better preservation of OM on the inner-shelf would be reflected in greater abundances of the more functionalized pheophytins and pyropheophytins than chlorophyllone (Fig. 4). This suggests that the transformation of chlorophyll *a* to chlorophyllone, pheophytin and pyropheophytin and the relative abundances are set in the Peru margin water-column, and that the reactivity of the three most abundant chlorins in the ambient geochemical conditions are similar.

The decrease in chlorin concentration between 200 and 400 m is consistent with degradation associated with resuspension, transport and extended residence time of OM at or above the sediment/water interface. These data are consistent with S1 + S2 values that decrease by a factor of 6 at depths between 200 and 400m (Fig. 4). It is surprising that chlorin degradation is most significant in the core of the OMZ, and that chlorin concentrations display little change where bottom waters become oxic below 600m. It is

possible that that remaining fraction of extractable chlorins is bound in proto-kerogen or protected from degradation by other mechanisms such as mineral association (e.g. Hedges and Kiel, 1995)

At low concentrations of dissolved oxygen ($< 5 \mu\text{M}$), the action of both aerobic and anaerobic heterotrophs within the OMZ is sufficient for extensive degradation of tetrapyrroles, a process that is clearly aided by reworking of OM. In the few samples for which pheophytin and pyropheophytin were below detection limits, chlorophyllone remained in low concentrations (Fig. 4). This could be attributed to some measure of recalcitrance of the chlorophyllone structure to mildly oxidizing conditions, or perhaps its preferential preservation is associated with packing in fecal pellets during herbivory.

The decrease in chlorin concentration at 200 m water depth is not directly associated with a large drop in productivity at the time of sample collection (Pancost et al., 1997). Application of the Chlorin Index (CI) to the geologic record has been utilized for characterizing changes in surface water phototrophic productivity (Higginson et al., 2003). However, in sediments where there are large changes in preservation downcore, particularly associated with variability in current activity on glacial-interglacial timescales (Ganeshram et al., 1999), application of the CI could be misleading.

The maximum concentration of chlorins in Peru Margin shelf sediments ($660 \text{ nmol} \cdot \text{gTOC}^{-1}$) is lower than the average porphyrin concentrations from mid-Cretaceous black shales of the Demerara Rise ($1285 \text{ nmol} \cdot \text{gTOC}^{-1}$) (Junium et al., 2008). We hypothesize that the primary difference between the two locations is related directly to oxidant exposure time. Oxygen deprivation characterized both settings, but the mid-Cretaceous deep-water over Demerara Rise was episodically euxinic which limited bioturbation and there is little evidence for strong bottom current. Without resuspension and reintroduction of OM into the water-column where oxidants such as sulfate are plentiful, preservation potential of tetrapyrroles is clearly greater, even considering the long time-scale of post burial diagenesis for Cretaceous strata.

4.4 Paleoenvironmental Implications

Recognizing the influence of strong bottom currents in the geologic record is extremely important for the interpretation of geochemical records from shelf and upper-slope sediments in upwelling environments. Ganeshram et al., (1999) present down-core

data from the Northwest Mexican margin that demonstrate the impact of sediment remobilization on the outer-shelf. Reductions in HI, OM burial and preservation are attributed winnowing by bottom currents evident by a lack of laminations and larger grain size (Ganeshram et al., 1999). The coring location is situated on the outer shelf (424 m water depth) below the region of modern undercurrent activity that is observed in other areas of the Eastern North Pacific, where flow is focused above 400 m with maximum flows found at 250 m (Lynn and Simpson, 1990). Eustatic sea-level lowering associated with northern hemisphere glaciation could have lowered the core of the undercurrent and shifted the primary zone of winnowing to the position of the modern outer-shelf. This mechanism could explain the decreases observed by Ganeshram et al., (1999) in OM preservation on outer-shelf of the Mexican Margin core during glacial intervals. If the diagenetic model that we have proposed is correct, the magnitude of the decrease in $\delta^{15}\text{N}$ of the Mexican outer-shelf could be in part the result of OM degradation and not decreasing denitrification (Ganeshram et al., 2002). Down-core compound-specific $\delta^{15}\text{N}$ analysis provides a method for testing this hypothesis in future studies.

These findings may also complicate efforts to reconstruct ancient algal ecology using ϵ_{por} when comparing $\delta^{15}\text{N}_{\text{bulk}}$ to $\delta^{15}\text{N}_{\text{chlorins}}$ in the manner of Higgins et al. (2012). When relying upon $\delta^{15}\text{N}_{\text{bulk}}$ for establishing ϵ_{por} values it is extremely important to have a clear understanding of processes that could potentially influence $\delta^{15}\text{N}_{\text{bulk}}$ values, whether that is diagenetic alteration of water column-derived OM or benthic productivity.

5 Conclusions

Dynamic sedimentary environments have the potential to significantly alter paleoceanographic proxies despite conditions that seem likely to provide excellent preservation. Data from two depth transects down the Peru Margin raise important questions about resistance of $\delta^{15}\text{N}_{\text{bulk}}$ values from surface sediments. Lateral transport increases the residence time of OM at or above the sediment water interface, allowing for significant degradation despite reducing conditions. Compound-specific $\delta^{15}\text{N}$ analyses of chlorophyll derivatives demonstrate that the ^{15}N -abundance of phototrophic N in surface sediments does not decrease offshore as is observed in $\delta^{15}\text{N}_{\text{bulk}}$. These data suggest that OM is sourced from the shelf and transported downslope, and that bulk $\delta^{15}\text{N}$ values were

altered during transport and reworking or reflect signals from multiple sources or more ancient organic matter. We would suggest that future studies constrain sampling to areas that are inboard of the shelf break and not influenced by bottom currents or receive a large flux of transported material. Additional factors such as addition of abundant microbial biomass (e.g. *Thioploca*), further complicate interpretation of bulk $\delta^{15}\text{N}$ data. The complexities and questions associated with bulk OM preservation and the multiple sources of OM present in sediments suggests that selective use of compound-specific methods provides a powerful approach to support conclusions drawn on bulk $\delta^{15}\text{N}$ analyses. The concentration of chlorophyll derivatives in Peru Margin sediments is significantly lower than the average concentration chlorophyll derivatives in some Cretaceous black shales. This fact illustrates the influence of lateral transport of sediment and degradation of OM and the effect of oxidants (FeOOH , NO_3^- , SO_4^{2-}) under anoxic conditions.

The issues raised in this dataset can be addressed by new approaches and new techniques that can help elucidate the controls on $\delta^{15}\text{N}_{\text{bulk}}$ in similar environments. A full appraisal of the concentration and nitrogen isotopic composition of a wide range of N-containing sedimentary fractions such as amino acids and mineral-associated N would help elucidate the influences of OM degradation. Radiocarbon dating techniques, especially compound specific $\Delta^{14}\text{C}$ on chlorophyll derivatives (Kusch et al., 2010) and amino acids may help resolve the source of the different chlorins and potential age disparities between chlorins and bulk organic matter.

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8 Figure Captions

Fig. 1. Site location of 1991-1992 Peru Margin Cruise

Fig. 2. HPLC UV/Vis chromatogram (665 μM) of a typical acetone extract. Sample 3354 3 from 183m water depth (15.3 % TOC).

Fig. 3. Detail of normal phase purification step for chlorophyllone (highlighted peaks) under isocratic elution conditions. Panel A is the UV/Vis chromatogram of a fraction of peaks 1 and 2 collected from reversed phase effluent and purified under normal phase. Early eluting compounds are primarily carotenoids that coelute with chlorophyllone. Panel B is the integrated LC-MS mass spectrum for the highlighted region that was collected for compound specific isotope analysis. The secondary peak at m/z 515 is the result of the loss of the hydroxyl group at C-15² from chlorophyllone.

Fig. 4. Bulk geochemical and chlorin concentration data from the 12°S (filled symbols) and 13.5°S location (open symbols) versus depth.

Fig. 5. Scatter plots of bulk geochemical data with respect to bulk $\delta^{15}\text{N}$. The locations at 12°S is represented by filled symbols and the 13.5°S location open symbols.

Fig. 6. Compound specific $\delta^{15}\text{N}_{\text{chlorin}}$ data from surface sediment samples all from the 12°S location. Corresponding $\delta^{15}\text{N}_{\text{bulk}}$ data from surface sediments are from the 12°S (filled symbols) and 13.5°S location (open symbols) versus depth. Error bars on $\delta^{15}\text{N}_{\text{chlorin}}$ are +/- 1‰.

Figure 1

Figure 2

Figure 3

Figure 6